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(54) Recombinant soluble Fc receptors

(57) Recombinant soluble Fc receptors according to the present invention are characterized by the absence of transmembrane domains, signal peptides and glycosylation. Such Fc receptors can easily be obtained by expressing respective nucleic acids in prokaryotic host cells and renaturation of the obtained inclusion bodies, which procedure leads to a very homogenous and pure product.

The products can be used for diagnostic as well as pharmaceutical applications and also for the generation of crystal structure data. Such crystal structure data can be used for the modelling of artificial molecules.

A further embodiment comprises coupling the Fc receptors according to the invention to solid materials like chromatography materials that can be used to separate and/or enrich antibodies.

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Description

[0001] The present invention relates to recombinant soluble Fc receptors (FcR), recombinant nucleic acids coding for such Fc receptors, host cells containing corresponding nucleic acids as well as a process for the determination of the amount of antibodies of a certain type contained in the blood, plasma or serum of a patient, a process for the determination of the immune status of patients with chronic diseases of the immune system and a process for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors. Further, the present invention is concerned with pharmaceutical compositions containing the recombinant soluble FcRs, the use of a crystalline preparation of recombinant soluble FcRs for the generation of crystal structure data of Fc receptors as well as FcR inhibitors and pharmaceutical compositions containing such FcR inhibitors.

[0002] A still further subject of the present invention is a recombinant Fc receptor coupled to a solid phase, e.g. a chromatography carrier material. The use of such chromatography material, which is another subject of the present invention, lies in the absorption of immunoglobulins from a body fluid of patients or from culture supernatants of immunoglobulin producing cells.

[0003] Fc receptors (FcRs) play a key role in defending the human organism against infections. After pathogens have gained access to the blood circulation they are opsonized by immunoglobulins (Igs). The resulting immunocomplexes bind due to their multivalency with high avidity to FcR bearing cells leading to clustering of the FcRs, which triggers several effector functions (Metzger, H., 1992A). These include, depending on the expressed FcR type and associated proteins, endocytosis with subsequent neutralization of the pathogens and antigen presentation, antibody-dependent cellular cytotoxity (ADCC), secretion of mediators or the regulation of antibody production (Fridman et al, 1992; van de Winkel and Capel, 1993).

[0004] Specific FcRs exist for all Ig classes, the ones for IgG being the most abundant with the widest diversity. Together with the high affinity receptor for IgE (FcεRIa), FcγRI (CD64), FcγRII (CD32) and FcγRIIIa (CD16) occur as type I transmembrane proteins or in soluble forms (sFcRs) but also a glycosylphosphatidylinositol anchored form of the FcγRIII (FcγRIIIb) exists. Furthermore, FcγRs occur in various isoforms (FcγRIa, b1, b2, c; FcγRIIa 1-2, b1-3, c) and alleles (FcγRIIa1-HR, -LR; FcγRIIIb-NA1,-NA2) (van de Winkel and Capel, 1993). In contrast to the overall homologous extracellular parts, the membrane spanning and the cytoplasmic domains differ. They may be deleted entirely or be of a size of 8 kDa. They may contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM) as in FcγRIIa or a respective 13 amino acid inhibitory motif (ITIM) in FcγRIIb involved in signal transduction (Amigorena et al, 1992).

[0005] Judged by the conserved spacing of cysteins, the extracellular part of the FcRs consists of three (FcγRI, CD64) or two (FcεRI, FcγRII, CD32 and FcγRIII, CD16) Ig-like domains (10 kDa/domain) and therefore belongs to the immunoglobulin super family. These highly glycosylated receptors are homologues, and the overall identity in amino acid sequence among the FcγRs and FcεRIa exceeds 50% in their extracellular regions. Nevertheless, the affinity of FcRs to their ligands varies widely. The higher affinity of $\approx 10^8 M^{-1}$ of the FcγRI to Fc-fragment is assigned to its third domain, while the other FcγRs with two domains have an affinity to IgG varying between 10^5 and $10^7 M^{-1}$. The affinity of the two domain FcεRIa to IgE exceeds these values by far with a constant of $10^{10} M^{-1}$ (Metzger, H., 1992B).

FcγRs are expressed in a defined pattern on all immunological active cells. FcγRI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils and eosinophils. The physiological role of FcγRI is still unknown as the expression on monocytes is not vital (Ceuppens et al, 1988). The GPI anchored form of FcγRIII (FcγRIIIb) is exclusively expressed on granulocytes. Due to its missing cytoplasmic part, the signal transduction into the cell occurs solely via other transmembrane proteins like complement receptor type 3 (CR3) that can at least associate with FcγRIIIb (Zhou et al, 1993; Poo et al, 1995). FcγRIIIa is mainly expressed on monocytes and macrophages but only in conjunction with associated proteins (e.g. α- or γ-chains). FcγRII is the receptor with the widest distribution on immunocompetent cells and is mainly involved in the endocytosis of immunocomplexes.

[0006] FcγRIIa and FcγRIIb differ in their extracellular region by only 7% of the amino acid residues. Nevertheless, both forms can be distinguished by their binding characteristics to human and mouse IgG subclasses (van de Winkel and Capel, 1993) and their differing affinity to human IgGs (Sondermann et al, 1998A). The situation is rendered even more complicated by the high responder/low responder (HR/LR) polymorphism of FcγRIIa named after the ability of T cells from some individuals to respond to murine IgG1-induced mitogenesis (Tax et al, 1 983). Later, it was found that the two exchanges in the amino acid sequence between the LR and the HR form modify the ability to bind human IgG2, which leads to the suggestion that at least one of them is involved in IgG binding (Hogarth et al, 1992).

[0007] In contrast to the beneficial role FcRs play in the healthy individual, they also transmit the stimulation of the immune system in allergies (FcεRIa) or autoimmune diseases. Moreover, some viruses employ FcγRs to get access to cells like HIV (Homsy et al, 1989) and Dengue (Littaua et al, 1990) or slow down the immune response by blocking FcγRs as in the case of Ebola (Yang at al, 1998) and Measles (Ravanel et al, 1997).

[0008] Hence, the object underlying the present invention was to provide receptors which are easy to produce and can advantageously be used for medical or diagnostic applications. Moreover, it was an object of the invention to pro-

vide soluble receptors exhibiting a binding specificity and activity which is analogous to that of the receptors occurring naturally in the human body and which, additionally, make it possible to produce crystals suitable for a structure determination.

[0009] This object is accomplished by recombinant soluble Fc receptors which consist only of the extracellular portion of the receptor and are not glycosylated. The receptors according to the present invention are therefore characterized by the absence of transmembrane domains, signal peptides and glycosylation.

[0010] Particularly preferred for the present invention are Fcγ or Fcε receptors. This is because IgG and IgE molecules are characteristic for a multiplicity of diseases and conditions, so that their determination and possible ways of influencing them are of great interest. Figure 8 shows an alignment of amino acid sequences of the extracellular parts of some FcγRs and FcεRI. The FcRs according to the invention include all these sequences or parts thereof that still retain binding capacity to antibodies and/or proper crystallization.

[0011] In a particularly preferred embodiment of the invention the recombinant soluble FcR is a FcγRIIb receptor. Further, it is particularly preferred that the receptor be of human origin. In a particularly preferred embodiment, it contains an amino acid sequence as shown in SEQ ID NO:1 or SEQ ID NO:2.

[0012] According to the present invention, the preparation of the soluble Fc receptors preferably takes place in prokaryotic cells. After such expression, insoluble inclusion bodies containing the recombinant protein form in prokaryotic cells, thus facilitating purification by separation of the inclusion bodies from other cell components before renaturation of the proteins contained therein takes place. The renaturation of the FcRs according to the present invention which are contained in the inclusion bodies can principally take place according to known methods. The advantage of the preparation in prokaryotic cells, the production of inclusion bodies and the thus obtained recombinant soluble Fc receptors make it possible to obtain a very pure and, in particular, also very homogeneous FcR preparation. Also because of the absence of glycosylation the obtained product is of great homogeneity.

[0013] Soluble Fc receptors hitherto produced by recombinant means particularly exhibited the disadvantage that a much more elaborate purification was required, since they were expressed in eukaryotic cells and, due to the glycosylation which is not always uniform in eukaryotic cells, these products were also less homogeneous.

[0014] The recombinant soluble Fc receptors according to the present invention even make it possible to produce crystals suitable for use in X-ray analysis, as shall be explained lateron. The FcRs of the present invention moreover exhibit practically the same activity and specificity as the receptors naturally occurring in vivo.

[0015] A further subject matter of the present invention is a recombinant nucleic acid having a sequence coding for a recombinant soluble Fc receptor according to the present invention.

[0016] The nucleic acid according to the present invention may contain only the coding sequences or, additionally, vector sequences and, in particular, expression control sequences operatively linked to the sequence encoding the recombinant FcR, like promoters, operators and the like.

[0017] In a particularly preferred embodiment the nucleic acid of the present invention contains a sequence as shown in SEQ ID NO:3 or SEQ ID NO:4.

[0018] In these sequence protocols the atg start codons are in bold print and newly introduced restriction sites are underlined. For a comparison, SEQ ID NO:5 and SEQ ID NO:6 show the respective wild type sequences coding for FcγRIIb and FcεRIa. SEQ ID NOs:7-9 show the wild type sequences for FcγRI, FcγRIIa and FcγRIII, which can be modified in a similar way as SEQ ID NO:3 and SEQ ID NO:4 to obtain FcRs according to the invention.

[0019] If the nucleic acid of the present invention contains vector sequences, then these are preferably sequences of one or several prokaryotic expression vectors, preferably of pET vectors. Any other known functions of expression vectors may also be contained in the recombinant nucleic acid according to the present invention if desired. These may, for instance, be resistance genes allowing for an effective selection of transformed host cells.

[0020] A still further subject matter of the present invention is a host cell containing a recombinant nucleic acid according to the present invention. As repeatedly mentioned above, the host cell preferably is a prokaryotic host cell, particularly an E. coli cell.

[0021] The recombinant soluble Fc receptors according to the present invention can be used for a multitude of examinations or applications because they specifically react with antibodies. In vivo, the soluble Fc receptors are powerful immunoregulators which, if present in elevated levels, result in a remarkable suppression of the immune system which leads to many partly known and partly not yet understood effects. Based on these effects, several applications of the Fc receptors according to the present invention are further subject matters of the present invention.

[0022] One such subject is a process for the determination of the amount of antibodies of a certain type in the blood or serum of a patient, which is characterized by the use of a recombinant soluble FcR according to the invention in an immunoassay, and the determination of the presence of FcR-antibody complexes. Such assay allows to screen for the presence of a certain kind of antibody and allows also for the determination of the amount of antibodies present in the blood, plasma or serum of a patient.

[0023] Any type of immunoassay is principally suitable for the use according to the present invention, as long as the presence of FcR-antibody complexes can thereby be detected. Both ELISA (enzyme-linked immunosorbent immu-

noassay), particularly sandwich assays, and RIA (radio-immunoassay) are suitable, but also competitive testing methods. In a preferred embodiment of the invention where the presence and/or the amount of IgE antibodies is to be examined, an FcεR is used as recombinant soluble receptor according to the present invention. In particular, this method is suited and advantageous for determining a predisposition or manifestation of an allergy.

[0024] Moreover, a method is preferred in which the presence of soluble FcRs is to be determined and, if required, quantified, an FcγR being used as recombinant soluble receptor according to the invention. By means of this test among others the immune status of patients with chronic diseases of the immune system can be determined in a competitive immunoassay. Chronic diseases in the sense of these processes are for instance AIDS, SLE (systemic lupus erythematosus), MM (multiple myeloma) or rheumatoid arthritis.

[0025] A further advantageous use of the receptor according to the present invention lies in the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

[0026] By means of modern screening techniques such as HTPS (high throughput screening) in combination with multi-well microtiter plates and automatic pipetting apparatuses it is nowadays possible to simultaneously test a multitude of substances for specific properties. As the FcRs according to the present invention can be easily produced at low cost, they can also be used in such series tests by which substances having an inhibiting effect can easily be identified.

[0027] Particularly preferred is such use according to which Fc receptors according to the present invention are used to find inhibitors capable of inhibiting the recognition and binding of the respective antibodies to the particular receptor of interest.

[0028] A further area of application of the substances according to the invention lies in the pharmaceutical field. Hence, a further subject matter of the invention is a pharmaceutical composition comprising as active agent a recombinant soluble FcR according to the invention. According to the present invention, this pharmaceutical composition may of course comprise conventional useful carrier and auxiliary substances. Such substances are known to the person of skill in the art, the mode of administration also having to be taken into account. The pharmaceutical composition of the present invention can be advantageously used for the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

[0029] Soluble forms of Fc receptors such as FcγRIII mediate isotype-specific regulation of B cell growth and immunoglobulin production. In a murine model of myeloma, sFcR suppresses growth and immunoglobulin production of tumor cells (Müller et al, 1985; Roman et al, 1988; Teillaud et al, 1990). Furthermore, sFcR binds to surface IgG on cultures of human IgG-secreting myeloma cells and effects suppression of tumor cell growth and IgG secretion. Prolonged exposure of these cells to sFcR results in tumor cell cytolysis (Hoover et al, 1995).

[0030] Also, overreactions of the immune system in allergic reactions or due to massive antigen load might be reduced by, for example, intravenous application of soluble FcR (Ierino et al, 1993).

[0031] Therefore, a preferred pharmaceutical composition according to the invention for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma contains a recombinant soluble Fcγ receptor and, preferably, a receptor having the amino acid sequence as shown in SEQ ID NO:1.

[0032] It was also of great interest to obtain crystal structure data of Fc receptors. On the one hand, these are a key to the understanding of molecular mechanisms in immunocomplex recognition. On the other hand, these structural data can be used to find out common features in the structures of different Fc receptors and use the knowledge of the structures to generate inhibitors or identify and produce new artificial antibody receptors.

[0033] To obtain such crystal structure data, a crystalline preparation of the recombinant soluble Fc receptor according to the invention is used. The recombinant soluble FcRs according to the invention surprisingly can be obtained pure enough to produce crystals that give reliable X-ray structure determination data. Such crystallization was not possible with the hitherto produced receptor molecules, mostly due to their lack of homogeneity.

[0034] The stated applications are merely preferred embodiments of the use of the crystal structure data. Many other applications seem possible, too.

[0035] Suitably, the structural data for the generation and/or identification of inhibitors or new receptors, respectively, are used in a computer-aided modelling program. Software for computer-aided modelling is available to the man skilled in the art. That application is already described for structure identification or design of other substances.

[0036] Particularly preferred for the present invention are the structures as shown in the enclosed Examples and Figures for the respective receptors. Such structures can be used to design inhibitors, antagonists and artificial receptor molecules.

[0037] A still further subject matter of the present invention, therefore, is a FcR inhibitor which has a three-dimensional structure which is complementary to the recombinant soluble FcR according to the invention and inhibits the binding of antibodies to FcRs.

[0038] What is important for the inhibitors of the invention is that, owing to their structure and specificity, they are capable of binding to the FcRs and thus prevent their normal binding to the constant parts of antibodies.

[0039] Preferably, such FcR inhibitors are small organic molecules which can easily be administered orally. They

might be an interesting alternative to cortisone in the treatment of autoimmune diseases and host/graft rejections. Such a molecule would also suppress reinfection rates with certain viruses, e.g. Dengue virus where the antibody coated virus is FcγRIIb dependent internalized (Littaua et al, 1990), HIV where on CD4 positive T cells an antibody enhancement of HIV infection is mediated by FcγRIII (Homsy et al, 1989), or Ebola where the virus secreted glycoprotein inhibits early neutrophil activation by blocking sFcγRIII which affects the host response to infection (Yang et al, 1998).

[0040] The development of inhibitors might also lead to substances that interfere with the recognition of IgE by their receptors. From the modelled structure of FcεRI, peptides have already been developed which inhibit mast cell degranulation in vitro. With the knowledge of the structures of the receptor or the receptor-antibody complex in atomic detail, a new possibility for a rational drug design is opened.

[0041] A further subject matter of the present invention therefore is a pharmaceutical composition containing as active agent an FcR inhibitor as mentioned above. Such pharmaceutical compositions may, for example, be used in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system, preferably the treatment or prevention of allergies, autoimmune diseases or anaphylactic shock.

[0042] A further subject of the present invention is the sFcR according to the invention, bound to a solid phase. Such heterogeneous receptors might be used for immunoassays or other applications where the receptor in an immobilized form can be used beneficially.

[0043] In a preferred embodiment of the invention the solid phase is a chromatography carrier material onto which the Fc receptor is fixed, e.g. sepharose, dextransulfate etc. Such chromatography materials with Fc receptors bound thereto can beneficially be used for the adsorption of immunoglobulins from the blood, plasma or serum of patients or from the culture supernatant of immunoglobulin producing cells (meaning concentration, enrichment and purification of antibodies).

[0044] On the one hand, the antibodies bound to the chromatography material can be eluted and, for example, the immune status of a patient can thereby be determined. On the other hand, antibodies from the blood of a patient can thereby be enriched before carrying out further tests, which is a further preferred embodiment of the present invention. In many cases it is difficult to conduct diagnostic assays using blood samples if the latter contains only a very small number of the antibodies to be identified. By means of a concentration using a specific chromatographic column with Fc receptors according to the present invention, antibodies of interest can easily be concentrated and separated from many other substances which might disturb the test.

[0045] Basically, it is also possible to use a chromatography material according to the present invention in an extracorporeal perfusion system for lavage of the blood in case of certain diseases where the removal of antibodies plays a crucial role.

[0046] The following Examples are to further illustrate the invention in conjunction with the Figures.

Example 1

1.1 Cloning and Expression

[0047] The cDNA of human FcγRIIb2 (Engelhardt et al, 1990) was modified using mutagenous PCR (Dulau et al, 1989). Therefore, a forward primer was used for the introduction of a new start methionine after the cleavage site of the signal peptide within a *NcoI* site (5'-AAT AGA ATT CCA TGG GGA CAC CTG CAG CTC CC-3') while the reverse primer introduced a stop codon between the putative extracellular part and the transmembrane region followed by a *SalI* site (5' CCC AGT GTC GAC AGC CTA AAT GAT CCC C-3'). The PCR product was digested with *NcoI* and *SalI*, cloned into a pET11d expression vector (Novagen) and the proposed sequence was confirmed. The final construct was propagated in BL21 (DE3) (Grodberg and Dunn, 1988). For the overexpression of FcγRIIb a single colony of the transformed bacteria was inoculated in 5ml LB medium containing 100 µg ampicillin per ml (LB-Amp100) and incubated overnight at 37°C. The culture was diluted 200-fold in LB-Amp100 and incubation was continued until an OD600 of 0.7-0.9 was achieved. The overproduction of the protein was induced by adding IPTG to a final concentration of 1 mM. After a growing period of 4 hours the cells were harvested by centrifugation (30 mm, 4000 x g) and resuspended in sonification buffer (30 mM sodium phosphate, 300 mM sodium chloride, 0.02% sodium azide, pH 7.8). After addition of 0.1 mg lysozyme per ml suspension and incubation for 30 min at room temperature the sonification was performed on ice (Branson Sonifier, Danbury, CT; Macrotip, 90% output, 80% interval, 15 min). The suspension was centrifuged (30 min, 30,000 x g) and resuspended with a Dounce homogenizer in sonification buffer containing 0.5% LDAO. The centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The purified inclusion bodies were stored at 4°C.

1.2 Refolding and purification of soluble human FcγRIIb (shFcγRIIb)

[0048] The purified inclusion bodies were dissolved to a protein concentration of 10 mg/ml in 6 M guanidine chlo-

ride, 100 mM 2-mercaptoethanol and separated from the insoluble matter by centrifugation. The refolding was achieved by rapid dilution. Therefore, one ml of the inclusion body solution was dropped under stirring within 15 hours into 400 ml of the refolding buffer (0.1 M TRIS/HCl, 1.4 M arginine, 150 mM sodium chloride, 5 mM GSH, 0.5 mM GSSG, 0.1 mM PMSF, 0.02% sodium azide, pH 8.5, 4°C). Afterwards, the mixture was stirred for 2-3 days until the concentration of free thiol groups was reduced to 1 mM by air oxidation as measured according to Ellman (Ellman, 1959). The solution was dialyzed against PBS and sterile filtered before it was concentrated 10-fold in a stirring cell equipped with a 3kD MWCO ultrafiltration membrane. The protein solution was applied to a hIgG sepharose column (50 mg hIgG per ml sepharose 4B). Unbound protein was washed out with 50 mM TRIS pH 8.0 before elution of FcγRIIb by pH jump (150mM sodium chloride, 100mM glycine, 0.02% sodium azide, pH 3.0). The eluate was immediately neutralized with 1 M TRIS pH 8.0. The FcγRIIb containing solution was concentrated and subjected to gel filtration on a Superdex-75 column equilibrated with crystallization buffer (2 mM MOPS 150 mM sodium chloride, 0.02% sodium azide pH 7.0). The fractions containing FcγRIIb were pooled, concentrated to 7 mg/ml and stored at -20°C.

1.3 Equilibrium gel filtration experiments

[0049] A Superdex75 column was connected to FPLC and equilibrated with PBS containing 10 µg shFcRIIb per ml. Human Fc fragment was solved to a concentration of 1 µg/10 µl in the equilibration buffer and injected. The resulting chromatogram yielded a positive peak comprising the complex of the shFcγRIIb and the Fc fragment while the negative peak represents the lack of receptor consumed from the running buffer for complex formation.

1.4 Crystallization and data collection

[0050] Initial crystallization trials employing a 96 condition sparse matrix screen (Jancarik and Kim, 1991) were performed in sitting drops at 20°C using the vapor diffusion method. Occuring crystals were improved by changing the pH as well as the salt, precipitant and additive concentration. Diffraction data from suitable crystals was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM (Leslie, 1997) and subsequently the data was scaled, reduced and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

1.5 Summary of expression, purification and refolding of shFcγRIIb

[0051] The extracellular part of FcγRIIb was expressed in high levels under the control of a T7 promoter in the T7 RNA polymerase positive E. coli strand BL21/DE3 (Grodberg & Dunn, 1988). The protein was deposited in inclusion bodies, which were employed in the first purification step. The isolation of the inclusion bodies was started with an intense combined lysozyme/ sonification procedure to open virtually all cells which would otherwise contaminate the product. The subsequent washing steps with the detergent LDAO, which has excellent properties in solving impurities but not the inclusion bodies itself already yielded a product with a purity of >90% (Fig. 1).

[0052] This product was used for refolding trials without further purification. The inclusion bodies were dissolved in high concentration of 2-mercaptoethanol and guanidine to ensure the shift of covalent and non-covalent aggregates to monomers. This solution was rapidly diluted with refolding buffer to minimize contacts between the unfolded protein molecules which would otherwise form aggregates. The use of arginine in the refolding buffer prevents the irreversible modification of side chains as often recognized with urea. After addition of the protein to the refolding buffer, the solution was stirred at 4°C until the concentration of free thiol groups was reduced to 1 mM, which was absolutely necessary as earlier dialysis resulted in an inactive product. In a second purification step the dialyzed and refolded FcγRIIb was bound to immobilized hIgG to remove minor fractions of E. coli proteins and inactive receptor. The protein was eluted with a pH jump and immediately neutralized. After this affinity chromatography step shFcγRIIb is essentially pure except for a minor contamination resulting from the coeluting IgG which leached out of the matrix even after repeated use (Fig. 1). The IgG as well as receptor multimers which are not visible in the reducing SDS-PAGE could easily be removed by gel filtration. Parallel to the removal of the contaminants in this step the buffer is quantitatively exchanged. This procedure ensures a defined composition of the protein solution as even slight variations can cause irreproducibility of the crystallization attempts or even inhibit the formation of crystals. Overall 6 mg pure protein could be gained per litre E. coli culture, which is about 10 % from the FcγRIIb content of the inclusion bodies.

[0053] N-terminal protein sequencing revealed the identity with the expected sequence H_2N-GTPAAP without detectable contamination. ESI-MS analysis showed that the final material used in crystallization trials is homogenous with respect to size. From the primary sequence the molecular weight was calculated to 20434 Da, which corresponds to 20429 Da found by mass spectroscopy. The discrepancy lies within the error of the instrument, and no additional peak for a species containing the leading methionine is found.

[0054] The crystallization of shFcγRIIb was performed in sitting drops using the vapor diffusion method. Initial trials with a sparse matrix screen (Jancarik & Kim, 1991) resulted already in small crystalline needles. Subsequent optimization of the preliminary crystallization condition by varying precipitant, salt, their concentration and pH led to the isolation of three different crystal forms. Orthorhombic crystals grew from mixture of 1.5 µl reservoir solution (33% PEG2000, 0.2 M sodium acetate, pH 5.4) with 3 µl of the protein solution. They appeared within 3 days and reached their final size of approximately 80µm x 80µm x 500µm after one week. These crystals diffracted to 1 .7 Å. Crystals could also be grown in two other space groups from reservoir solution containing 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM $Zn(OAc)_2$, 100 mM sodium chloride (hexagonal form) and 26% PEG8000, 0.2 M NaOAc, pH 5.6, 10% (v/v) 1,4-Dioxan, 100mM sodium chloride (tetragonal form). These crystals were of suitable size for X-ray analysis but diffracted only to 2.7 Å and 3.8 Å for the tetragonal and hexagonal crystal form respectively (Table 1).

[0055] FcγRII was expressed in E. coli which, besides the comparatively low production costs and the availability, has several advantages especially when the glycosylation performed by mammalian cells is not necessary for the function of the protein as in the case of FcγRII where IgG binding occurs independently of carbohydrate attachment (Sondermann et al, 1998A). In E. coli a homogenous product can reproducibly be generated, which is in contrast to the expression in mammalian cells where batch dependent variances are often observed. In such a system the product is for several days exposed to proteases at temperatures of more than 30°C. In contrary, the expression of the protein in E. coli under the control of the strong T7 promoter at 37°C frequently leads to the formation of protease inaccessible inclusion bodies. A further advantage of the expression in bacteria is that the material could be considered to be free of pathogenic germs, which might derive from employed fetal calf serum or the cell line itself. In mammalian expression particular care must be taken during the purification of the target protein because potential effective hormones or growth factors might be copurified. One case where the effects of sFcγR were ascribed to a TGFβ1 contamination is already reported (Galon et al, 1995).

1.6 Purification

[0056] The purification procedure is straightforward. It consists of three steps which can easily be performed in a single day. The protein is obtained in a pure form and in high yields and could even be obtained in considerable quality without the expensive IgG affinity column. The success of such a protocol would depend on the careful preparation of the inclusion bodies, as most of the impurities can be eliminated already in the first purification step.

1.7 Characterization

[0057] The purified FcγRIIb was characterized by SDS-PAGE and isoelectric focussing as well as N-terminal sequencing and mass spectroscopy. Thus, the material can be considered pure and homogeneous with respect to its chemical composition, but the intriguing question whether the receptor is correctly folded remains to be discussed. All cysteins are paired, since no free thiol groups are detected with Ellman's test. The material is monomeric and eludes with the expected retention time in peaks of symmetrical shape from a size exclusion chromatography column. Furthermore, FcγRIIb binds to IgG sepharose, recombinant FcγRIIb from E. coli is active because it specifically binds IgG.

1.8 Crystallization

[0058] The orthorhombic crystal form of FcγRIIb diffracted X-rays to a resolution of 1.7 Å, which is a drastic improvement compared to previously reported crystals of the same molecule derived from insect cell expression (Sondermann et al, 1998A). These crystals diffracted to 2.9 Å and were of space group $P3_121$. Thus, the glycosylation of the insect cell derived receptor influences the crystallization conditions. Instead of the trigonal space group, three different crystal forms are found. After a possible solution of the structure these crystal forms will help identify artificial conformations of the protein due to crystal contacts.

[0059] FcγRs do not exhibit any sequence similarity to other proteins but due to a conserved cystein spacing they are affiliated to the immunoglobulin super family. Consequently, we tried to solve its structure by molecular replacement, but extensive trials using IgG domains from a variety of molecules failed. Thus the structure of FcγRIIb has to be solved by the methods of multiple isomorphous replacement.

[0060] We have shown for the first time that FcγRIIb can be obtained in an active form from E. coli. This is the basis for crystallographic investigations that will soon, due to the already gained crystals of exceptional quality, result in the structure solution of this important molecule. The structure will provide information on the IgG binding site and provide a starting point for the knowledge based design of drugs that interfere with recognition of the ligand by its receptor. Furthermore, because of the high homology between FcγRIIb and other FcRs including FcεRIa it seems possible that these molecules can be produced in the same way, which would provide valuable material for the ongoing research.

Example 2

2.1 Methods

Protein chemistry

[0061] Recombinant soluble human FcγRIIb was expressed in E.coli, refolded purified and crystallized as described elsewhere (Sondermann et al, 1998B). Briefly, the putative extracellular region of hFcγRIIb2 (Engelhardt et al, 1990) was overexpressed in E. coli. Inclusion bodies were purified by lysozyme treatment of the cells and subsequent sonification. The resulting suspension was centrifuged (30 min 30,000 x g) and washed with buffer containing 0.5% LDAO. A centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were solved in 6 M guanidine hydrochloride and the protein was renaturated as described. The dialyzed and filtrated protein solution was applied to a hIgG sepharose column and eluted by pH jump. The concentrated neutralized fractions were subjected to size-exclusion chromatography on a Superdex-75 column (26/60, Pharmacia).

Crystallization

[0062] Crystallization was performed in sitting drops at 20°C using the vapor diffusion technique. Crystallization screens were performed by changing pH, salt, precipitant and additives. The final crystals used for data collection were grown in 33% PEG2000, 0.2 M sodium acetate, pH 5.4 (orthorhombic form) 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v) 1,4-dioxane, 100 mM sodium chloride (tetragonal form), and 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5mM $ZN(OAc)_2$, 100mM sodium chloride (hexagonal form). The insect cell derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

Preparation of heavy-atom derivatives

[0063] The heavy-atom derivatives were prepared by soaking the crystals in the crystallization buffer containing 2 mM platinum(II)-(2,2'-6,2"terpyridinium) chloride for 24 hours or 10 mM uranylchloride for 8 days.

X-ray data collection

[0064] Diffraction data was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM 5.50 (Leslie, 1997) and subsequently the data was scaled and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

Structure determination

[0065] The structure was solved with the standard procedures of the MIR method. From the large number of soaks carried out with different heavy-atom components only the two compounds yielded interpretable Patterson maps. The heavy-atom positions for each derivative were determined from difference Patterson maps and initial phases were calculated. Cross-phased difference Fourier maps were used to confirm heavy atom positions and establish a common origin for the derivatives. Anomalous data were included to discriminate between the enantiomers. The heavy atom parameters were further refined with the program MLPHARE from the CCP4 package leading to the statistics compiled in Table 2. An electron-density map was calculated to a resolution of 2.1 Å and the phases were improved further by solvent flattening and histogram matching with the program DM from the CCP4 suite. The resulting electron density map was of sufficient quality to build most of the amino acid residues. Model building was performed with O (Jones et al, 1991) on an Indigo2 work station (Silicon Graphics Incorporation). The structure refinement was done with XPLOR (Brünger et al, 1987) by gradually increasing the resolution to 1.7 Å using the parameter set of Engh and Huber (Engh & Huber, 1991). When the structure was complete after several rounds of model building and individual restraint B-factors refinement (R_{fac} = 29% / R_{Free} = 36%), 150 water molecules were built into the electron density when a Fo-Fc map contoured at 3.5 σ coincided with well defined electron density of a 2Fo-Fc map contoured at 1 σ. The resulting refinement statistic is shown in Table 3.

2.2 Structure determination

[0066] The crystal structure of recombinant soluble human FcγRIIb was solved by multiple isomorphous replacement (MIR) to 1.7 Å resolution, since a structure solution by molecular replacement with isolated domains of the Fc fragment from human IgG1 (Huber et al, 1976, PDB entry 1fc1; Deisenhofer, 1981) failed. The putative extracellular part of the receptor (amino acid residues 1-187 as depicted in SEQ ID NO:2) was used for crystallization trials (Sondermann et al, 1998B) while the model contains the residues 5-176 as the termini are flexible and not traceable into the electron density. Additionally, the model contains 150 water molecules and the refinement statistics are summarized in Table 2. The structure contains a cis proline at position 11. None of the main chain torsion angles is located in disallowed regions of the Ramachandran plot. The fully refined model was used to solve the structure of the same protein in crystals of space group $P4_22_12$ and of the glycosylated form derived from insect cells in crystals of space group $P3_121$ (Table 2).

[0067] The polypeptide chain of FcγRIIb folds into two Ig-like domains as expected from its affiliation with the immunoglobulin super family. Each domain consists of two beta sheets that are arranged in a sandwich with the conserved disulfide bridge connecting strands B and F on the opposing sheets (Fig. 3). Three anti-parallel β-strands (A1, B, E) oppose a sheet of 5 β-strands (C', C, F, G, A2), whereby strand A1 leaves the 3-stranded β-sheet and crosses over to the 4-stranded anti-parallel sheet adding the short parallel 5th strand A2. The arrangement of secondary structure elements as well as their connectivity is identical in both domains of the FcγRIIb and a rigid body fit of one domain onto the other revealed a r.m.s. distance of 1 .29 Å of 67 matching Cα atoms.

[0068] The domains are arranged nearly perpendicularly to each other enclosing an angle of 70 degrees between their long axes forming a heart-shaped overall structure. This arrangement results in an extensive contact region between the domains (Fig. 4). Residues from strand A2 and from the segment linking A2 and A1 of the N-terminal domain intermesh with residues of strands A1 and B from the C-terminal domain. This region is tightly packed and the interaction is strengthened by several hydrogen bonds resulting in a rigid arrangement. This is confirmed by the conservation of the structure in three different space groups. In orthorhombic, tetragonal and hexagonal (insect cell derived) crystal forms a deviation of less than 2° in the interdomain angle is found.

2.3 Overall structures

[0069] The structure of recombinant human FcγRIIb derived from E.coli was solved by MIR to 1.7 Å resolution from orthorhombic crystals. An essentially identical structure is found in tetragonal and with protein derived from insect cells in hexagonal crystals. In all three structures the last nine residues of the polypeptide chain were found disordered. The flexibility of the C-terminal linker region between the structured core of the molecule and the transmembrane part may be functionally relevant to allow some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

2.4 Homologue receptors

[0070] The Ig domains found in the Ig super family of proteins are characterized by a beta sandwich structure with a conserved disulfide bridge connecting two strands of the opposing sheets. The typical arrangement of 3 and 4 anti parallel beta strands that form a sandwich as found in FcγRIIb occurs also in the T cell receptor, Fc fragment, CD4 or the Fab fragment. A structural alignment of the individual Ig domains of these molecules with the two domains of FcγRIIb shows a common, closely related structure. The relative arrangement of the domains, however, is not related in these molecules and covers a broad sector. Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of Cα atoms that result when the two domains of FcγRII are superimposed, no significant sequence similarity is found (Figs. 5a and 5b). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains, together with, beside the cysteins, only few identical amino acid residues. We first prepared a structure-based alignment of the two C-terminal domains of the IgG1 heavy chain and the FcγRIIb and added the sequences of the other related FcγR and the FcεRIa domains. This shows that the sequences of the three domain FcγRI and the two domain receptors are compatible with the hydrophobicity pattern of Ig domains and several conserved amino acid residues are revealed. Firstly, the different domains of an FcR are more related to each other than to Ig domains from other molecules of the Ig super family. Secondly, the N-terminal domains of the receptors relate to each other as the second domains do. Thirdly, the sequence of the third domain of FcγRI shows features from both groups of domains. Taken together, we confirm the affiliation of the FcRs to the Ig super family and speculate that all FcR-domains originate from a common ancestor, an ancient one domain receptor that acquired a second domain by gene duplication. Further divergent development of such a two domain receptor resulted in the present diversity, including FcγRI that acquired a third domain.

[0071] Conservation of these amino acid residues that contribute to the interdomain contact in FcγRIIb in the align-

ment are a hint to a similar domain arrangement in different receptors. In Table 4 the residues contributing with their side chains to the interdomain contact (Fig. 4) are compiled for FcγRIIb together with the corresponding amino acid residues in other receptors according to the structure-based sequence alignment of Fig. 5b. Except for Asn15, which is not conserved between the FcRs, the involved residues are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs.

2.5 The contact interface to IgG

[0072] Limited information about the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth et al, 1992; Hulett et al, 1994; Hulett et al, 1995). By systematically exchanging loops between the β-strands of FcγRIIa for FcεRIa amino acid residues the B/C, C'/E and F/G loops of the C-terminal domain were evaluated as important for ligand binding (Fig. 3, Fig. 5b). In the structure model these loops are adjacent and freely accessible to the potential ligand. Additionally, most of the amino acid residues in these loops were exchanged for alanines by single site mutations which resulted in a drastic alteration of the affinity of FcγRIIa to dimeric human IgG1. Also, the single amino acid exchange Arg 131 to His in the C-terminal domain (C'/E loop) in the high responder/low responder polymorphism, which alters the affinity of the FcγRIIa to murine IgG1, points to that region. Thus, the amino acid residues in this area are either important for ligand binding or the structural integrity of that region. Here, the structure shows a clustering of the hydrophobic amino acid residues Pro 114, Leu 115 and Val 116 in the neighbourhood of Tyr 157. This patch is separated from the region Leu 159, Phe 121 and Phe 129 by the positively charged amino acid residues Arg 131 and Lys 117 which protrude from the core structure (Fig. 5b).

2.6 Glycosylation

[0073] In the sequence of FcγRIIb three potential N-glycosylation sites are found. All three sites are on the surface of the molecule and are accessible. They are located in the E/F loops (N61 and N142) of both domains and on strand E (N135) of the C-terminal domain (Fig. 3, Fig. 6). Since the material used for the solution of this structure was obtained from E. coli, it does not contain carbohydrates, while the FcRs isolated from mammalian cells are highly glycosylated. The three potential glycosylation sites are located rather far from the putative IgG binding region, and non-glycosylated FcγRIIb binds human IgG, suggesting a minor role of glycosylation in binding. This was confirmed by the structure of the FcγRIIb produced in insect cells which is glycosylated (Sondermann et al, 1998A). Except for a 2° change of the interdomain angle possibly due to different crystal contacts, no differences between the glycosylated and unglycosylated protein structures were found. The three glycosylation sites are only optionally used as shown by SDS-PAGE where the material appears in 4 bands. No additional electron density for those sugars was found a consequence of chemical and structural heterogeneity.

2.7 The modeled complex

[0074] The newly solved structure of FcγRIIb complements the information gained from the structure of the Fc fragment and the available biochemical data regarding the FcγR:IgG complex.

[0075] While diverse biochemical information concerning the binding site of FcγRIIb (see above) is available, only limited data exists regarding the contact area contributed by the antibody. The IgG isotypes are closely related and exhibit graded affinities to FcγRs. However, they still carry too many amino acid exchanges for the determination of the binding site and the preparation of IgG mutants is tedious. The only available information results from experiments with FcγR bearing cells on which bound immunocomplexes could be displaced with protein A (Ades et al, 1976), suggesting an at least partially overlapping binding site of protein A and FcγRIIb on the antibody.

[0076] With the structures of both constituents at hands we attempted to model the FcγRII:IgG complex using the program FTDock (Gabb et al, 1997). FTDock uses Fourier correlation theory for evaluation of the shape and electrostatic complementarity of the complex component surfaces. In the hands of the authors the program has produced good results in predicting complex structures, but in some cases additional biochemical information on the location of the contact area was needed to exclude false positive solutions.

[0077] Without applying additional restrictions concerning the region of the contact surface between FcγRIIb and the Fc fragment, the calculations resulted in a single solution clearly scoring above the rather constant background. The program predicted a complex structure with the B/C, C'/E and F/G loops of the FcγRIIb domain 2 contributing to the contact site as predicted by the mutagenesis experiments. The only observed interaction of the N-terminal domain with the Fc fragment is via E19 that forms a salt bridge to a lysine of the CH_2 domain. Some involvement of residues of the N-terminal domain in complex formation is expected since the N-terminal domain of FcγRIIa cannot be exchanged against the corresponding domain of FcεRIa (Hulett et al, 1995) without losing the ligand binding capability of the receptor.

[0078] From the predicted interaction a model of the membrane bound complex between IgG and FcγRIIb is pro-

posed (Fig. 7). Two FcγRIIb bind into the cleft between the third and the fourth domain of the IgG heavy chains employing the 2-fold symmetry of the Fc fragment. Protein A (Deisenhofer et al, 1978; Deisenhofer, 1981) as well as protein G (Sauer-Eriksson et al, 1995) and the neonatal FcR (Burmeister et al, 1994) bind to a surface region around the exposed hydrophobic residue Ile 253 of the Fc fragment. FcγRIIb binds to a region in the vicinity consistent with the competitive binding of protein A and FcγR to the antibody. The 2:1 stoichiometry between FcγRIIb and Fc fragment in the complex could be shown in equilibrium gel filtration experiments (Sondermann et al, 1998A).

[0079] The complex can be positioned upright on the membrane, with the truncated C-termini of FcγRIIb oriented towards the membrane. The N-terminal domain of the receptor lies parallel to the membrane between the Fab arms when the complex is viewed along the Fc fragment. If the FcRs have evolved from a common one domain receptor we expect that the amino acid residues of the N-terminal domain that correspond to the binding region of the C-terminal domain form a second putative binding site. The corresponding surface region is accessible in the proposed complex and forms a large uncharged patch with a hydrophobic ridge comprising amino acid residues Pro 47, Leu 45, Phe 40, Leu 75, Pro 3, Pro 2 and Ala 1 (Fig. 6b). This region might represent a binding site for other ligands that have been discussed for FcγRIIb to explain the signalling capabilities of its soluble form.

[0080] Thus the modeled complex structure is consistent with the available biochemical data.

Fig. 1: 15% reducing SDS PAGE showing the purification of sFcγRIIb
Lane 1: Molecular weight marker. Lane 2: E. coli lysate before induction. Lane 3: E. coli lysate 1 h after induction. Lane 4: E.coli lysate 4 h after induction. Lane 5: Purified inclusion bodies of sFcγRIIb. Lane 6: Eluate of the hIgG affinity column. Lane 7: Pooled fractions of the gel filtration column.

Fig. 2: Equilibrium gel filtration
1 µg hFc solved in 10µl equilibration buffer (10 µg sFcγRIIb/ml PBS) was applied to a size exclusion chromatography column and the absorbance of the effluent was measured (280 nm) as a function of time. The injected Fc fragment forms a complex with the sFcγRIIb in the equilibration buffer (t = 22min). The negative peak of consumed sFcγRIIb is observed at t = 26 min.

Fig. 3: Overall structure of human sFcγRIIb
Stereo ribbon representation of the sFcγRIIb structure. The loops supposed to be important for IgG binding are depicted in red with some of the residues within the binding site and the conserved disulfide bridge in ball and stick representation. The potential N-glycosylation sites are shown as green balls. The termini are labeled and the β-strands are numbered consecutively for the N-terminal domain in black and for the C-terminal domain in blue. The figure was created using the programs MOLSCRIPT (Kraulis, 1991) and RENDER (Merritt and Murphy, 1994).

Fig. 4: Interdomain contacts
The figure shows a close-up on the residues involved in the interdomain contacts of sFcγRIIb. The amino acid residues of the N-terminal domain are depicted blue and the residues of the C-terminal domain yellow. The model is covered by a 2Fo-Fc electron density contoured at 1 σ obtained from the final coordinates. Hydrogen bridges between the domains are represented by white lines. The figure was created using the program MAIN (Turk, 1992).

Fig. 5a: Superposition of the two FcγRIIb domains and the CH2 domain of human IgG1
Both domains of FcγRIIb and the CH2 domain of hIgG1 were superimposed. The N-terminal domain is depicted in blue, the C-terminal domain in red and the CH2 domain of hIgG1 in green. The respective termini are labeled and the conserved disulfide bridges are depicted as thin lines.

Fig. 5b: Structure based sequence alignment of the sFcγRIIb domains with domains of other members of the FcR family
The upper part of the figure shows the structure based sequence alignment of the FcγRIIb and hIgG1 Fc fragment domains performed with the program GBF-3D-FIT (Lessel & Schomburg, 1994). Amino acid residues with a Cα distance of less than 2.0 Å in the superimposed domains are masked: lilac for matching residues between the Fc fragment domains; yellow for residues in the FcγRIIb domains; and green when they can be superimposed in all four domains. The β-strands are indicated below this part of the alignment and are labeled consistent with Figure 3. The lower part of the figure shows the alignment of the amino acid sequences from the other FcγRs and the homologue FcεRIa to the profile given in the upper part of the figure using routines from the GCG package (Genetics Computer Group, 1994). The upper and lower row of numbering refer to the N- and C-terminal domains of FcγRIIb. The conserved cysteins are typed in magenta and the potential glycosylation sites in blue. Identical residues within the first domain are masked orange, those in the second domain pink and green when the residues are conserved within both domains. The less conserved third domain of FcγRI is aligned between the first and the second

domains. Red arrows point to residues that are involved in side chain contacts between the first and the second domain while blue arrows depict residues that are relevant for IgG binding. The figure was produced with the program ALSCRIPT (Barton, 1993).

Fig. 6: The putative binding sites of FcγRIIb

Solid surface representations of FcγRIIb as produced with GRASP (Nicholls et al, 1991), the color coding is according to the relative surface potential from negative (red) to positive (blue). Fig. 6a shows the molecule as in Fig. 3 by a rotation of about 90° counter-clockwise around the vertical. In Fig. 6b the molecule is rotated 90° clockwise around the same axis. Both views show the putative binding regions on the C-terminal (Fig. 6a) and the N-terminal domain (Fig. 6b). The amino acid residues discussed in the text are labeled.

Fig. 7: Model of the FcγR-IgG complex

The cartoon shows a complete complex of two FcγRIIb binding one antibody as suggested by the program FFT-DOCK. The heavy chains of the antibody are depicted in red and green and the light chains in yellow. The blue atoms represent the C-terminal domain of sFcγRIIb while the white ones represent the N-terminal domain. A blue column connects the receptor to the membrane instead of the flexible linker region that remained invisible in the electron density. The image was produced with the program POVRAY.

Fig. 8: Alignment of the amino acid sequence of the extracellular parts of FcγR and FcεRIa

Figure 8 shows an alignment of amino acid sequences of the extracellular parts of some FcγRs and FcεRI.

Table 1

Crystallographic results			
The obtained preliminary crystallographic data are shown in this table.			
	Orthorhombic	**Tetragonal**	**Hexagonal**
Space group	$P2_12_12_1$ [19]	$P4_22_12$ [94]	P3 [143]
Unit cell dimensions	a=40.8Å,b=50.9Å, c=80.5Å, α=90°, β=90°,γ=90°	a=85.7Å,b=85.7Å, c=63.4Å, α=90°, β=90°,γ=90°	a=80.9Å,b=80.9Å c=157.0Å, α=90°, β=90°,γ=90°
R_{merge}	5.8%	9.8%	13.6%
Resolution	1.7Å	2.7Å	3.8Å
Unique	18,040	6,616	7,210
Completeness	89.1%	97.1%	63.0%
Multiplicity	3.5	4.4	1.3
V_M, molecules per asymmetric unit, solvent content	2.09Å^3/Da, 1mol., 41 % solvent	2.91Å/Da, 1 mol, 58% solvent	2.97Å/Da, 5 mol, 59% solvent

Table 2: Data collection statistics

Derivative	Space Group	No. of unique reflections	Multi-plicity	Resolution (Å)	Complete-ness (overall/ last shell) (%/%)	R_m (%)	No. of sites	Phasing power
NATI	$P2_12_12_1$.	18009	3.6	1.74	92.9/86.4	5.5		
NATI	$P4_12_12$	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	P3,21	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	$P2_12_12_1$.	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPy	$P2_12_12_1$.	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$R_m = \Sigma|I_h - \langle I_h \rangle| / \Sigma \langle I_h \rangle$

Phasing power: $\langle F_H \rangle / E$, where $\langle F_H \rangle = \Sigma(F_H^2/n)^{1/2}$ is the r.m.s. heavy atom structure amplitude.

$E = \Sigma[(F_{PHC} - F_{PH})^2/n]^{1/2}$ is the residual lack of closure error with F_{PH} being the structure factor amplitude and $F_{PHC} = |F_P + F_H|$ the calculated structure factor amplitude of the derivative.

Table 3

Refinement statistics	
Resolution range (Å)	8.0 - 1.74 Å
No. of unique reflections ($F>O\sigma$ (F))	16252
R factor	19.4
R_{free}*	27.9
No. of atoms per asymmetric unit	
protein	1371
solvent	150
Rms deviation from ideal geometry	
bond length (Å)	0.009
bond angle (°)	2.007
Average B factors ($Å^2$)	
protein main chain	18.8
protein side chain	25.2
solvent	36.7
Rms deviation of bonded B factors ($Å^2$)	4.1

*R_{free} :5% of the reflections were used as a reference data set and were not included in the refinement.

Table 4

Residues that contribute to the interdomain contact via side chains				
FcγRIIb	FcγRIIa	FcγRIII	FcγRI	FcεRIa
Asn15	Asn	Ser	Ser	Arg
Asp20	Asp	Asp	Glu	Glu
Gln91	Gln	Gln	Gln	Gln
His108	His	His	His	His
Trp110	Trp	Trp	Trp	Trp

SEQ ID NO:1

GTPAAPPKAV LKLEPQWINV LQEDSVTLTC RGTQSPESDS IQWFHNGNLI PTHTQPSYRF
KANNNDSGEY TCQTGQTSVS DPVHLTVLSE WLVLQTPHLE FQEGETIVLR CHSWKDKPLV
KVTFFQNGKS KKFSRSDPNF SIPQANHSHS GDYHCTGNIG YTLYSSKPVT ITVQAP...S
SSPMGII

SEQ ID NO:2

AVPQKPK VSLNPPWNRI PKGENVTLTC NGNNFFEVSS TKWFHNGSLS EETNSSLNIV
NAKFEDSGEY KCQHQQVNES EPVYLEVFSD WLLLQASAEV VMEGQPLFLR CHGWRNWDVY
KVIYYKDGEA LKYWYENHNI SITNATVEDS GTYYCTGKVW QLDYESEPLN ITVIKAPREK
YWLQ(F)

SEQ ID NO:3

aacagaa ttccATGggg acacctgcag

181 ctcccccaaa ggctgtgctg aaactcgagc cccagtggat caacgtgctc caggaggact
241 ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
301 acaatgggaa tctcattccc acccacacgc agcccagcta caggttcaag gccaacaaca
361 atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac cctgtgcatc
421 tgacagtgct ttctgagtgg ctggtgctcc agacccctca cctggagttc caggagggag
481 aaaccatcgt gctgaggtgc cacagctgga aggacaagcc tctggtcaag gtcacattct
541 tccagaatgg aaaatccaag aaattttccc gttcggatcc caacttctcc atcccacaag
601 caaaccacag tcacagtggt gattaccatt gcacaggaaa cataggctac acgctgtact
661 catccaagcc tgtgaccatc actgtccaag ctcccagctc ttcaccgatg gggatcattT
721 AGgctgtcga cactggg

SEQ ID NO:4

```
                                                    gatggccata tggcagtccct
121   cagaaaccta aggtctcctt gaaccctcca tggaatagaa tatttaaagg agagaatgtg
181   actcttacat gtaatgggaa caatttcttt gaagtcagtt ccaccaaatg gttccacaat
241   ggcagccttt cagaagagac aaattcaagt ttgaatattg tgaatgccaa atttgaagac
301   agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa
361   gtcttcagtg actggctgct ccttcaggcc tctgctgagg tggtgatgga gggccagccc
421   ctcttcctca ggtgccatgg ttggaggaac tgggatgtgt acaaggtgat ctattataag
481   gatggtgaag ctctcaagta ctggtatgag aaccacaaca tctccattac aaatgccaca
541   gttgaagaca gtggaaccta ctactgtacg ggcaaagtgt ggcagctgga ctatgagtct
601   gagcccctca acattactgt aataaaagct ccgcgtgaga agtactggct acaattttag
661   gatccattg
```

SEQ ID NO:5

```
human FcγRIIb2
1     ggctgtgact gctgtgctct gggcgccact cgctccaggg agtgatggga atcctgtcat
61    ttttacctgt ccttgccact gagagtgact gggctgactg caagtccccc cagccttggg
121   gtcatatgct tctgtggaca gctgtgctat tcctggctcc tgttgctggg acacctgcag
181   ctcccccaaa ggctgtgctg aaactcgagc cccagtggat caacgtgctc caggaggact
241   ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
301   acaatgggaa tctcattccc acccacacgc agcccagcta caggttcaag gccaacaaca
361   atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac cctgtgcatc
421   tgacagtgct ttctgagtgg ctggtgctcc agacccctca cctggagttc caggagggag
481   aaaccatcgt gctgaggtgc cacagctgga aggacaagcc tctggtcaag gtcacattct
541   tccagaatgg aaaatccaag aaattttccc gttcggatcc caacttctcc atcccacaag
601   caaaccacag tcacagtggt gattaccatt gcacaggaaa cataggctac acgctgtact
661   catccaagcc tgtgaccatc actgtccaag ctcccagctc ttcaccgatg gggatcattg
721   tggctgtggt cactgggatt gctgtagctg ccattgttgc tgctgtagtg gccttgatct
781   actgcaggaa aaagcggatt tcagccaatc ccactaatcc tgatgaggct gacaaagttg
841   gggctgagaa cacaatcacc tattcacttc tcatgcaccc ggatgctctg gaagagcctg
901   atgaccagaa ccgtatttag tctccattgt cttgcattgg gatttgagaa gaaatcagag
961   agggaagatc tggtatttcc tggcctaaat tccccttggg gaggacaggg agatgctgca
1021  gttccaaaag agaaggtttc ttccagagtc atctacctga gtcctgaagc tccctgtcct
1081  gaaagccaca gacaatatgg tcccaaatgc ccgactgcac cttctgtgct tcagctcttc
1141  ttgacatcaa ggctcttccg ttccacatcc acacagccaa tccaattaat caaaccactg
1201  ttattaacag ataatagcaa cttgggaaat gcttatgtta caggttacgt gagaacaatc
1261  atgtaaatct atatgatttc agaaatgtta aaatagacta acctctacca gcacattaaa
1321  agtgattgtt tctgggtgat aaaattattg atgattttta ttttctttat ttttctataa
1381  agatcatata ttacttttat aataaaacat tataaaaac
```

SEQ ID NO:6

human FcεRIa

```
1     agatctcagc acagtaagca ccaggagtcc atgaagaaga tggctcctgc catggaatcc
61    cctactctac tgtgtgtagc cttactgttc ttcgctccag atggcgtgtt agcagtccct
121   cagaaaccta aggtctcctt gaaccctcca tggaatagaa tatttaaagg agagaatgtg
181   actcttacat gtaatgggaa caatttcttt gaagtcagtt ccaccaaatg gttccacaat
241   ggcagccttt cagaagagac aaattcaagt ttgaatattg tgaatgccaa atttgaagac
301   agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa
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421   ctcttcctca ggtgccatgg ttggaggaac tgggatgtgt acaaggtgat ctattataag
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ttc tat ctg gca gtg gga ata atg ttt tta gtg aac act gtt ctc tgg 966
Phe Tyr Leu Ala Val Gly Ile Met Phe Leu Val Asn Thr Val Leu Trp
295 300 305 310

gtg aca ata cgt aaa gaa ctg aaa aga aag aaa aag tgg gat tta gaa 1014
Val Thr Ile Arg Lys Glu Leu Lys Arg Lys Lys Lys Trp Asp Leu Glu
315 320 325

atc tct ttg gat tct ggt cat gag aag aag gta act tcc agc ctt caa 1062
Ile Ser Leu Asp Ser Gly His Glu Lys Lys Val Thr Ser Ser Leu Gln
330 335 340

gaa gac aga cat tta gaa gaa gag ctg aaa tgt cag gaa caa aaa gaa 1110
Glu Asp Arg His Leu Glu Glu Glu Leu Lys Cys Gln Glu Gln Lys Glu
345 350 355

gaa cag ctg cag gaa ggg gtg cac cgg aag gag ccc cag ggg gcc acg 1158
Glu Gln Leu Gln Glu Gly Val His Arg Lys Glu Pro Gln Gly Ala Thr
360 365 370

tagcagcggc tcagtgggtg gccatcgatc tggaccgtcc cctgcccact tgctccccgt 1218

gagcactgcg tacaaacatc caaaagttca acaacaccag aactgtgtgt ctcatggtat 1278

gtaactctta aagcaaataa atgaactgac ttcaaaaaaa aaa 1321

<210> 2
<211> 374
<212> PRT
<213> Homo sapiens

<400> 2
Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Ser Leu Gln Pro Pro Trp Val Ser
20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln

```
     50                  55                  60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
 65                  70                  75                  80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
                 85                  90                  95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
            100                 105                 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
        115                 120                 125

Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
    130                 135                 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145                 150                 155                 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
                165                 170                 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
            180                 185                 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
        195                 200                 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
    210                 215                 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225                 230                 235                 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
                245                 250                 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
            260                 265                 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro
        275                 280                 285

Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu
    290                 295                 300

Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys
```

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305                 310                 315                 320

Lys Lys Trp Asp Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys
                325                 330                 335

Val Thr Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Glu Leu Lys
            340                 345                 350

Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys
        355                 360                 365

Glu Pro Gln Gly Ala Thr
    370
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<210> 3
<211> 1419
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (45)..(917)

<400> 3

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ggctgtgact gctgtgctct gggcgccact cgctccaggg agtg atg gga atc ctg  56
                                                  Met Gly Ile Leu
                                                    1

tca ttt tta cct gtc ctt gcc act gag agt gac tgg gct gac tgc aag  104
Ser Phe Leu Pro Val Leu Ala Thr Glu Ser Asp Trp Ala Asp Cys Lys
  5                 10                  15                  20

tcc ccc cag cct tgg ggt cat atg ctt ctg tgg aca gct gtg cta ttc  152
Ser Pro Gln Pro Trp Gly His Met Leu Leu Trp Thr Ala Val Leu Phe
                25                  30                  35

ctg gct cct gtt gct ggg aca cct gca gct ccc cca aag gct gtg ctg  200
Leu Ala Pro Val Ala Gly Thr Pro Ala Ala Pro Pro Lys Ala Val Leu
            40                  45                  50

aaa ctc gag ccc cag tgg atc aac gtg ctc cag gag gac tct gtg act  248
Lys Leu Glu Pro Gln Trp Ile Asn Val Leu Gln Glu Asp Ser Val Thr
        55                  60                  65

ctg aca tgc cgg ggg act cac agc cct gag agc gac tcc att cag tgg  296
Leu Thr Cys Arg Gly Thr His Ser Pro Glu Ser Asp Ser Ile Gln Trp
    70                  75                  80
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ttc cac aat ggg aat ctc att ccc acc cac acg cag ccc agc tac agg   344
Phe His Asn Gly Asn Leu Ile Pro Thr His Thr Gln Pro Ser Tyr Arg
 85                  90                  95                 100

ttc aag gcc aac aac aat gac agc ggg gag tac acg tgc cag act ggc   392
Phe Lys Ala Asn Asn Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly
                105                 110                 115

cag acc agc ctc agc gac cct gtg cat ctg aca gtg ctt tct gag tgg   440
Gln Thr Ser Leu Ser Asp Pro Val His Leu Thr Val Leu Ser Glu Trp
            120                 125                 130

ctg gtg ctc cag acc cct cac ctg gag ttc cag gag gga gaa acc atc   488
Leu Val Leu Gln Thr Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile
        135                 140                 145

gtg ctg agg tgc cac agc tgg aag gac aag cct ctg gtc aag gtc aca   536
Val Leu Arg Cys His Ser Trp Lys Asp Lys Pro Leu Val Lys Val Thr
    150                 155                 160

ttc ttc cag aat gga aaa tcc aag aaa ttt tcc cgt tcg gat ccc aac   584
Phe Phe Gln Asn Gly Lys Ser Lys Lys Phe Ser Arg Ser Asp Pro Asn
165                 170                 175                 180

ttc tcc atc cca caa gca aac cac agt cac agt ggt gat tac cat tgc   632
Phe Ser Ile Pro Gln Ala Asn His Ser His Ser Gly Asp Tyr His Cys
                185                 190                 195

aca gga aac ata ggc tac acg ctg tac tca tcc aag cct gtg acc atc   680
Thr Gly Asn Ile Gly Tyr Thr Leu Tyr Ser Ser Lys Pro Val Thr Ile
            200                 205                 210

act gtc caa gct ccc agc tct tca ccg atg ggg atc att gtg gct gtg   728
Thr Val Gln Ala Pro Ser Ser Ser Pro Met Gly Ile Ile Val Ala Val
        215                 220                 225

gtc act ggg att gct gta gct gcc att gtt gct gct gta gtg gcc ttg   776
Val Thr Gly Ile Ala Val Ala Ala Ile Val Ala Ala Val Val Ala Leu
    230                 235                 240

atc tac tgc agg aaa aag cgg att tca gcc aat ccc act aat cct gat   824
Ile Tyr Cys Arg Lys Lys Arg Ile Ser Ala Asn Pro Thr Asn Pro Asp
245                 250                 255                 260

gag gct gac aaa gtt ggg gct gag aac aca atc acc tat tca ctt ctc   872
Glu Ala Asp Lys Val Gly Ala Glu Asn Thr Ile Thr Tyr Ser Leu Leu
                265                 270                 275
```

atg cac ccg gat gct ctg gaa gag cct gat gac cag aac cgt att 917
Met His Pro Asp Ala Leu Glu Glu Pro Asp Asp Gln Asn Arg Ile
280 285 290

tagtctccat tgtcttgcat tgggatttga gaagaaatca gagagggaag atctggtatt 977

tcctggccta aattcccctt ggggaggaca gggagatgct gcagttccaa aagagaaggt 1037

ttcttccaga gtcatctacc tgagtcctga agctccctgt cctgaaagcc acagacaata 1097

tggtcccaaa tgcccgactg caccttctgt gcttcagctc ttcttgacat caaggctctt 1157

ccgttccaca tccacacagc caatccaatt aatcaaacca ctgttattaa cagataatag 1217

caacttggga aatgcttatg ttacaggtta cgtgagaaca atcatgtaaa tctatatgat 1277

ttcagaaatg ttaaaataga ctaacctcta ccagcacatt aaaagtgatt gtttctgggt 1337

gataaaatta ttgatgattt ttatttttctt tatttttcta taaagatcat atattacttt 1397

tataataaaa cattataaaa ac 1419

<210> 4
<211> 291
<212> PRT
<213> Homo sapiens

<400> 4
Met Gly Ile Leu Ser Phe Leu Pro Val Leu Ala Thr Glu Ser Asp Trp
1 5 10 15

Ala Asp Cys Lys Ser Pro Gln Pro Trp Gly His Met Leu Leu Trp Thr
20 25 30

Ala Val Leu Phe Leu Ala Pro Val Ala Gly Thr Pro Ala Ala Pro Pro
35 40 45

Lys Ala Val Leu Lys Leu Glu Pro Gln Trp Ile Asn Val Leu Gln Glu
50 55 60

Asp Ser Val Thr Leu Thr Cys Arg Gly Thr His Ser Pro Glu Ser Asp
65 70 75 80

Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His Thr Gln
85 90 95

```
Pro Ser Tyr Arg Phe Lys Ala Asn Asn Asn Asp Ser Gly Glu Tyr Thr
            100                 105                 110

Cys Gln Thr Gly Gln Thr Ser Leu Ser Asp Pro Val His Leu Thr Val
        115                 120                 125

Leu Ser Glu Trp Leu Val Leu Gln Thr Pro His Leu Glu Phe Gln Glu
    130                 135                 140

Gly Glu Thr Ile Val Leu Arg Cys His Ser Trp Lys Asp Lys Pro Leu
145                 150                 155                 160

Val Lys Val Thr Phe Phe Gln Asn Gly Lys Ser Lys Lys Phe Ser Arg
                165                 170                 175

Ser Asp Pro Asn Phe Ser Ile Pro Gln Ala Asn His Ser His Ser Gly
            180                 185                 190

Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr Thr Leu Tyr Ser Ser Lys
        195                 200                 205

Pro Val Thr Ile Thr Val Gln Ala Pro Ser Ser Ser Pro Met Gly Ile
    210                 215                 220

Ile Val Ala Val Val Thr Gly Ile Ala Val Ala Ala Ile Val Ala Ala
225                 230                 235                 240

Val Val Ala Leu Ile Tyr Cys Arg Lys Lys Arg Ile Ser Ala Asn Pro
                245                 250                 255

Thr Asn Pro Asp Glu Ala Asp Lys Val Gly Ala Glu Asn Thr Ile Thr
            260                 265                 270

Tyr Ser Leu Leu Met His Pro Asp Ala Leu Glu Glu Pro Asp Asp Gln
        275                 280                 285

Asn Arg Ile
    290
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<210> 5
<211> 2359
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (6)..(938)

<400> 5

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cccaa atg tct cag aat gta tgt ccc aga aac ctg tgg ctg ctt caa cca 50
      Met Ser Gln Asn Val Cys Pro Arg Asn Leu Trp Leu Leu Gln Pro
        1               5                  10                  15

ttg aca gtt ttg ctg ctg ctg gct tct gca gac agt caa gct gca gct  98
Leu Thr Val Leu Leu Leu Leu Ala Ser Ala Asp Ser Gln Ala Ala Ala
                20                  25                  30

ccc cca aag gct gtg ctg aaa ctt gag ccc ccg tgg atc aac gtg ctc  146
Pro Pro Lys Ala Val Leu Lys Leu Glu Pro Pro Trp Ile Asn Val Leu
            35                  40                  45

cag gag gac tct gtg act ctg aca tgc cag ggg gct cgc agc cct gag  194
Gln Glu Asp Ser Val Thr Leu Thr Cys Gln Gly Ala Arg Ser Pro Glu
        50                  55                  60

agc gac tcc att cag tgg ttc cac aat ggg aat ctc att ccc acc cac  242
Ser Asp Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His
    65                  70                  75

acg cag ccc agc tac agg ttc aag gcc aac aac aat gac agc ggg gag  290
Thr Gln Pro Ser Tyr Arg Phe Lys Ala Asn Asn Asn Asp Ser Gly Glu
80                  85                  90                  95

tac acg tgc cag act ggc cag acc agc ctc agc gac cct gtg cat ctg  338
Tyr Thr Cys Gln Thr Gly Gln Thr Ser Leu Ser Asp Pro Val His Leu
                100                 105                 110

act gtg ctt tcc gaa tgg ctg gtg ctc cag acc cct cac ctg gag ttc  386
Thr Val Leu Ser Glu Trp Leu Val Leu Gln Thr Pro His Leu Glu Phe
            115                 120                 125

cag gag gga gaa acc atc atg ctg agg tgc cac agc tgg aag gac aag  434
Gln Glu Gly Glu Thr Ile Met Leu Arg Cys His Ser Trp Lys Asp Lys
        130                 135                 140

cct ctg gtc aag gtc aca ttc ttc cag aat gga aaa tcc cag aaa ttc  482
Pro Leu Val Lys Val Thr Phe Phe Gln Asn Gly Lys Ser Gln Lys Phe
    145                 150                 155

tcc cgt ttg gat ccc acc ttc tcc atc cca caa gca aac cac agt cac  530
Ser Arg Leu Asp Pro Thr Phe Ser Ile Pro Gln Ala Asn His Ser His
160                 165                 170                 175

agt ggt gat tac cac tgc aca gga aac ata ggc tac acg ctg ttc tca  578
Ser Gly Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr Thr Leu Phe Ser
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                180                 185                 190

tcc aag cct gtg acc atc act gtc caa gtg ccc agc atg ggc agc tct   626
Ser Lys Pro Val Thr Ile Thr Val Gln Val Pro Ser Met Gly Ser Ser
        195                 200                 205

tca cca atg ggg atc att gtg gct gtg gtc att gcg act gct gta gca   674
Ser Pro Met Gly Ile Ile Val Ala Val Val Ile Ala Thr Ala Val Ala
    210                 215                 220

gcc att gtt gct gct gta gtg gcc ttg atc tac tgc agg aaa aag cgg   722
Ala Ile Val Ala Ala Val Val Ala Leu Ile Tyr Cys Arg Lys Lys Arg
225                 230                 235

att tca gcc aat tcc act gat cct gtg aag gct gcc caa ttt gag cca   770
Ile Ser Ala Asn Ser Thr Asp Pro Val Lys Ala Ala Gln Phe Glu Pro
240                 245                 250                 255

cct gga cgt caa atg att gcc atc aga aag aga caa ctt gaa gaa acc   818
Pro Gly Arg Gln Met Ile Ala Ile Arg Lys Arg Gln Leu Glu Glu Thr
                260                 265                 270

aac aat gac tat gaa aca gct gac ggc ggc tac atg act ctg aac ccc   866
Asn Asn Asp Tyr Glu Thr Ala Asp Gly Gly Tyr Met Thr Leu Asn Pro
            275                 280                 285

agg gca cct act gac gat gat aaa aac atc tac ctg act ctt cct ccc   914
Arg Ala Pro Thr Asp Asp Asp Lys Asn Ile Tyr Leu Thr Leu Pro Pro
        290                 295                 300

aac gac cat gtc aac agt aat aac taaagagtaa cgttatgcca tgtggtcata  968
Asn Asp His Val Asn Ser Asn Asn
    305                 310

ctctcagctt gctgatggat gacaaaaaga ggggaattgt taaaggaaaa tttaaatgga 1028

gactggaaaa atcctgagca aacaaaacca cctggccctt agaaatagct ttaactttgc 1088

ttaaactaca aacacaagca aaacttcacg gggtcatact acatacaagc ataagcaaaa 1148

cttaacttgg atcatttctg gtaaatgctt atgttagaaa taagacaacc ccagccaatc 1208

acaagcagcc tactaacata taattaggtg actagggact ttctaagaag atacctaccc 1268

ccaaaaaaca attatgtaat tgaaaaccaa ccgattgcct ttattttgct tccacatttt 1328

cccaataaat acttgcctgt gacattttgc cactggaaca ctaaacttca tgaattgcgc 1388
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ctcagatttt tcctttaaca tctttttttt ttttgacaga gtctcaatct gttacccagg 1448

ctggagtgca gtggtgctat cttggctcac tgcaaacccg cctcccaggt ttaagcgatt 1508

cttatgcctc agcctcccag tagctgggat tagaggcatg tgccatcata cccagctaat 1568

ttttgtattt tttatttttt atttttagta gagacagggt ttcgcaatgt tggccaggcc 1628

gatctcgaac ttctggcctc tagcgatctg cccgcctcgg cctcccaaag tgctgggatg 1688

accgcatcag ccccaatgtc cagcctcttt aacatcttct ttcctatgcc ctctctgtgg 1748

atccctactg ctggtttctg ccttctccat gctgagaaca aaatcaccta ttcactgctt 1808

atgcagtcgg aagctccaga agaacaaaga gcccaattac cagaaccaca ttaagtctcc 1868

attgttttgc cttgggattt gagaagagaa ttagagaggt gaggatctgg tatttcctgg 1928

actaaattcc cttggggaag acgaagggat gctgcagttc caaaagagaa ggactcttcc 1988

agagtcatct acctgagtcc caaagctccc tgtcctgaaa gccacagaca atatggtccc 2048

aaatgactga ctgcaccttc tgtgcctcag ccgttcttga catcaagaat cttctgttcc 2108

acatccacac agccaataca attagtcaaa ccactgttat taacagatgt agcaacatga 2168

gaaacgctta tgttacaggt tacatgagag caatcatgta agtctatatg acttcagaaa 2228

tgttaaaata gactaacctc taacaacaaa ttaaaagtga ttgtttcaag gtgatgcaat 2288

tattgatgac ctatttttatt tttctataat gatcatatat tacctttgta ataaaacatt 2348

ataaccaaaa c 2359

<210> 6
<211> 311
<212> PRT
<213> Homo sapiens

<400> 6

Met Ser Gln Asn Val Cys Pro Arg Asn Leu Trp Leu Leu Gln Pro Leu
1 5 10 15

Thr Val Leu Leu Leu Leu Ala Ser Ala Asp Ser Gln Ala Ala Ala Pro
20 25 30

Pro Lys Ala Val Leu Lys Leu Glu Pro Pro Trp Ile Asn Val Leu Gln

```
        35                  40                  45

Glu Asp Ser Val Thr Leu Thr Cys Gln Gly Ala Arg Ser Pro Glu Ser
     50                  55                  60

Asp Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His Thr
 65                  70                  75                  80

Gln Pro Ser Tyr Arg Phe Lys Ala Asn Asn Asn Asp Ser Gly Glu Tyr
                 85                  90                  95

Thr Cys Gln Thr Gly Gln Thr Ser Leu Ser Asp Pro Val His Leu Thr
            100                 105                 110

Val Leu Ser Glu Trp Leu Val Leu Gln Thr Pro His Leu Glu Phe Gln
        115                 120                 125

Glu Gly Glu Thr Ile Met Leu Arg Cys His Ser Trp Lys Asp Lys Pro
    130                 135                 140

Leu Val Lys Val Thr Phe Phe Gln Asn Gly Lys Ser Gln Lys Phe Ser
145                 150                 155                 160

Arg Leu Asp Pro Thr Phe Ser Ile Pro Gln Ala Asn His Ser His Ser
                165                 170                 175

Gly Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr Thr Leu Phe Ser Ser
            180                 185                 190

Lys Pro Val Thr Ile Thr Val Gln Val Pro Ser Met Gly Ser Ser Ser
        195                 200                 205

Pro Met Gly Ile Ile Val Ala Val Val Ile Ala Thr Ala Val Ala Ala
    210                 215                 220

Ile Val Ala Ala Val Val Ala Leu Ile Tyr Cys Arg Lys Lys Arg Ile
225                 230                 235                 240

Ser Ala Asn Ser Thr Asp Pro Val Lys Ala Ala Gln Phe Glu Pro Pro
                245                 250                 255

Gly Arg Gln Met Ile Ala Ile Arg Lys Arg Gln Leu Glu Glu Thr Asn
            260                 265                 270

Asn Asp Tyr Glu Thr Ala Asp Gly Gly Tyr Met Thr Leu Asn Pro Arg
        275                 280                 285

Ala Pro Thr Asp Asp Asp Lys Asn Ile Tyr Leu Thr Leu Pro Pro Asn
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    290                 295                 300

Asp His Val Asn Ser Asn Asn
305                 310


<210> 7
<211> 887
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (34)..(732)

<400> 7
tctttggtga cttgtccact ccagtgtggc atc atg tgg cag ctg ctc ctc cca  54
                                     Met Trp Gln Leu Leu Leu Pro
                                       1               5

act gct ctg cta ctt cta gtt tca gct ggc atg cgg act gaa gat ctc   102
Thr Ala Leu Leu Leu Leu Val Ser Ala Gly Met Arg Thr Glu Asp Leu
         10                  15                  20

cca aag gct gtg gtg ttc ctg gag cct caa tgg tac agc gtg ctt gag   150
Pro Lys Ala Val Val Phe Leu Glu Pro Gln Trp Tyr Ser Val Leu Glu
     25                  30                  35

aag gac agt gtg act ctg aag tgc cag gga gcc tac tcc cct gag gac   198
Lys Asp Ser Val Thr Leu Lys Cys Gln Gly Ala Tyr Ser Pro Glu Asp
 40                  45                  50                  55

aat tcc aca cag tgg ttt cac aat gag agc ctc atc tca agc cag gcc   246
Asn Ser Thr Gln Trp Phe His Asn Glu Ser Leu Ile Ser Ser Gln Ala
                 60                  65                  70

tcg agc tac ttc att gac gct gcc aca gtc aac gac agt gga gag tac   294
Ser Ser Tyr Phe Ile Asp Ala Ala Thr Val Asn Asp Ser Gly Glu Tyr
             75                  80                  85

agg tgc cag aca aac ctc tcc acc ctc agt gac ccg gtg cag cta gaa   342
Arg Cys Gln Thr Asn Leu Ser Thr Leu Ser Asp Pro Val Gln Leu Glu
         90                  95                  100

gtc cat atc ggc tgg ctg ttg ctc cag gcc cct cgg tgg gtg ttc aag   390
Val His Ile Gly Trp Leu Leu Leu Gln Ala Pro Arg Trp Val Phe Lys
    105                 110                 115
```

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gag gaa gac cct att cac ctg agg tgt cac agc tgg aag aac act gct   438
Glu Glu Asp Pro Ile His Leu Arg Cys His Ser Trp Lys Asn Thr Ala
120                 125                 130                 135

ctg cat aag gtc aca tat tta cag aat ggc aaa gac agg aag tat ttt   486
Leu His Lys Val Thr Tyr Leu Gln Asn Gly Lys Asp Arg Lys Tyr Phe
                140                 145                 150

cat cat aat tct gac ttc cac att cca aaa gcc aca ctc aaa gat agc   534
His His Asn Ser Asp Phe His Ile Pro Lys Ala Thr Leu Lys Asp Ser
            155                 160                 165

ggc tcc tac ttc tgc agg ggg ctt gtt ggg agt aaa aat gtg tct tca   582
Gly Ser Tyr Phe Cys Arg Gly Leu Val Gly Ser Lys Asn Val Ser Ser
        170                 175                 180

gag act gtg aac atc acc atc act caa ggt ttg gca gtg tca acc atc   630
Glu Thr Val Asn Ile Thr Ile Thr Gln Gly Leu Ala Val Ser Thr Ile
    185                 190                 195

tca tca ttc tct cca cct ggg tac caa gtc tct ttc tgc ttg gtg atg   678
Ser Ser Phe Ser Pro Pro Gly Tyr Gln Val Ser Phe Cys Leu Val Met
200                 205                 210                 215

gta ctc ctt ttt gca gtg gac aca gga cta tat ttc tct gtg aag aca   726
Val Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe Ser Val Lys Thr
                220                 225                 230

aac att tgaagctcaa caagagactg gaaggaccat aaacttaaat ggagaaagga   782
Asn Ile

ccctcaagac aaatgacccc catcccatgg gagtaataag agcagtggca gcagcatctc 842

tgaacatttc tctggatttg caaccccatc atcctcaggc ctctc                887
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<210> 8
<211> 233
<212> PRT
<213> Homo sapiens

<400> 8

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Met Trp Gln Leu Leu Leu Pro Thr Ala Leu Leu Leu Leu Val Ser Ala
  1               5                  10                  15

Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
            20                  25                  30
```

```
Gln Trp Tyr Ser Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
         35                  40                  45

Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
     50                  55                  60

Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr
 65                  70                  75                  80

Val Asn Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu
                 85                  90                  95

Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Leu Gln
            100                 105                 110

Ala Pro Arg Trp Val Phe Lys Glu Glu Asp Pro Ile His Leu Arg Cys
        115                 120                 125

His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn
    130                 135                 140

Gly Lys Asp Arg Lys Tyr Phe His His Asn Ser Asp Phe His Ile Pro
145                 150                 155                 160

Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Val
                165                 170                 175

Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln
            180                 185                 190

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Ser Pro Pro Gly Tyr Gln
        195                 200                 205

Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr Gly
    210                 215                 220

Leu Tyr Phe Ser Val Lys Thr Asn Ile
225                 230
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<210> 9
<211> 1068
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (31)..(810)

<400> 9

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agatctcagc acagtaagca ccaggagtcc atg aag aag atg gct cct gcc atg  54
                                  Met Lys Lys Met Ala Pro Ala Met
                                    1               5

gaa tcc cct act cta ctg tgt gta gcc tta ctg ttc ttc gct cca gat  102
Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu Phe Phe Ala Pro Asp
     10                  15                  20

ggc gtg tta gca gtc cct cag aaa cct aag gtc tcc ttg aac cct cca  150
Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro
 25                  30                  35                  40

tgg aat aga ata ttt aaa gga gag aat gtg act ctt aca tgt aat ggg  198
Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly
                 45                  50                  55

aac aat ttc ttt gaa gtc agt tcc acc aaa tgg ttc cac aat ggc agc  246
Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser
             60                  65                  70

ctt tca gaa gag aca aat tca agt ttg aat att gtg aat gcc aaa ttt  294
Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe
         75                  80                  85

gaa gac agt gga gaa tac aaa tgt cag cac caa caa gtt aat gag agt  342
Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser
     90                  95                  100

gaa cct gtg tac ctg gaa gtc ttc agt gac tgg ctg ctc ctt cag gcc  390
Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala
105                 110                 115                 120

tct gct gag gtg gtg atg gag ggc cag ccc ctc ttc ctc agg tgc cat  438
Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His
                125                 130                 135

ggt tgg agg aac tgg gat gtg tac aag gtg atc tat tat aag gat ggt  486
Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly
            140                 145                 150

gaa gct ctc aag tac tgg tat gag aac cac aac atc tcc att aca aat  534
Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn
        155                 160                 165

gcc aca gtt gaa gac agt gga acc tac tac tgt acg ggc aaa gtg tgg  582
Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp
```

```
    170                 175                 180

cag ctg gac tat gag tct gag ccc ctc aac att act gta ata aaa gct   630
Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala
185                 190                 195                 200

ccg cgt gag aag tac tgg cta caa ttt ttt atc cca ttg ttg gtg gtg   678
Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val
                205                 210                 215

att ctg ttt gct gtg gac aca gga tta ttt atc tca act cag cag cag   726
Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Gln
            220                 225                 230

gtc aca ttt ctc ttg aag att aag aga acc agg aaa ggc ttc aga ctt   774
Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu
        235                 240                 245

ctg aac cca cat cct aag cca aac ccc aaa aac aac tgatataatt        820
Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn Asn
    250                 255                 260

aactcaagaa atatttgcaa cattagtttt tttccagcat cagcaattgc tactcaattg 880

tcaaacacag cttgcaatat acatagaaac gtctgtgctc aaggatttat agaaatgctt 940

cattaaactg agtgaaactg attaagtggc atgtaatagt aagtgctcaa ttaacattgg 1000

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ttagatct                                                          1068


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            20                  25                  30

Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu
        35                  40                  45
```

```
Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser
     50                  55                  60

Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser
 65                  70                  75                  80

Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys
                85                  90                  95

Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe
            100                 105                 110

Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly
        115                 120                 125

Gln Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr
    130                 135                 140

Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu
145                 150                 155                 160

Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr
                165                 170                 175

Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro
            180                 185                 190

Leu Asn Ile Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln
        195                 200                 205

Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala Val Asp Thr Gly
    210                 215                 220

Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu Leu Lys Ile Lys
225                 230                 235                 240

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Pro Lys Asn Asn
            260
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             Met Gly Thr Pro Ala Ala Pro Pro Lys Ala Val Leu Lys
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ctc gag ccc cag tgg atc aac gtg ctc cag gag gac tct gtg act ctg  98
Leu Glu Pro Gln Trp Ile Asn Val Leu Gln Glu Asp Ser Val Thr Leu
         15                  20                  25

aca tgc cgg ggg act cac agc cct gag agc gac tcc att cag tgg ttc  146
Thr Cys Arg Gly Thr His Ser Pro Glu Ser Asp Ser Ile Gln Trp Phe
     30                  35                  40

cac aat ggg aat ctc att ccc acc cac acg cag ccc agc tac agg ttc  194
His Asn Gly Asn Leu Ile Pro Thr His Thr Gln Pro Ser Tyr Arg Phe
 45                  50                  55                  60

aag gcc aac aac aat gac agc ggg gag tac acg tgc cag act ggc cag  242
Lys Ala Asn Asn Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly Gln
                 65                  70                  75

acc agc ctc agc gac cct gtg cat ctg aca gtg ctt tct gag tgg ctg  290
Thr Ser Leu Ser Asp Pro Val His Leu Thr Val Leu Ser Glu Trp Leu
             80                  85                  90

gtg ctc cag acc cct cac ctg gag ttc cag gag gga gaa acc atc gtg  338
Val Leu Gln Thr Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile Val
         95                  100                 105

ctg agg tgc cac agc tgg aag gac aag cct ctg gtc aag gtc aca ttc  386
Leu Arg Cys His Ser Trp Lys Asp Lys Pro Leu Val Lys Val Thr Phe
    110                 115                 120

ttc cag aat gga aaa tcc aag aaa ttt tcc cgt tcg gat ccc aac ttc  434
Phe Gln Asn Gly Lys Ser Lys Lys Phe Ser Arg Ser Asp Pro Asn Phe
125                 130                 135                 140

tcc atc cca caa gca aac cac agt cac agt ggt gat tac cat tgc aca  482
Ser Ile Pro Gln Ala Asn His Ser His Ser Gly Asp Tyr His Cys Thr
                145                 150                 155
```

```
gga aac ata ggc tac acg ctg tac tca tcc aag cct gtg acc atc act   530
Gly Asn Ile Gly Tyr Thr Leu Tyr Ser Ser Lys Pro Val Thr Ile Thr
            160                 165                 170

gtc caa gct ccc agc tct tca ccg atg ggg atc att tag gctgtcgaca   579
Val Gln Ala Pro Ser Ser Ser Pro Met Gly Ile Ile
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ctggg                                                            584
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             20                  25                  30

Gly Thr His Ser Pro Glu Ser Asp Ser Ile Gln Trp Phe His Asn Gly
         35                  40                  45

Asn Leu Ile Pro Thr His Thr Gln Pro Ser Tyr Arg Phe Lys Ala Asn
     50                  55                  60

Asn Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly Gln Thr Ser Leu
 65                  70                  75                  80

Ser Asp Pro Val His Leu Thr Val Leu Ser Glu Trp Leu Val Leu Gln
                 85                  90                  95

Thr Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile Val Leu Arg Cys
            100                 105                 110

His Ser Trp Lys Asp Lys Pro Leu Val Lys Val Thr Phe Phe Gln Asn
        115                 120                 125

Gly Lys Ser Lys Lys Phe Ser Arg Ser Asp Pro Asn Phe Ser Ile Pro
    130                 135                 140

Gln Ala Asn His Ser His Ser Gly Asp Tyr His Cys Thr Gly Asn Ile
145                 150                 155                 160
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               165                 170                 175

Pro Ser Ser Ser Pro Met Gly Ile Ile
            180                 185


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           -1   1               5                  10

tgg aat aga ata ttt aaa gga gag aat gtg act ctt aca tgt aat ggg   99
Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly
     15                  20                  25

aac aat ttc ttt gaa gtc agt tcc acc aaa tgg ttc cac aat ggc agc  147
Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser
 30                  35                  40                  45

ctt tca gaa gag aca aat tca agt ttg aat att gtg aat gcc aaa ttt  195
Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe
                 50                  55                  60

gaa gac agt gga gaa tac aaa tgt cag cac caa caa gtt aat gag agt  243
Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser
             65                  70                  75

gaa cct gtg tac ctg gaa gtc ttc agt gac tgg ctg ctc ctt cag gcc  291
Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala
         80                  85                  90

tct gct gag gtg gtg atg gag ggc cag ccc ctc ttc ctc agg tgc cat  339
Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His
     95                  100                 105
```

```
ggt tgg agg aac tgg gat gtg tac aag gtg atc tat tat aag gat ggt   387
Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly
110                 115                 120                 125

gaa gct ctc aag tac tgg tat gag aac cac aac atc tcc att aca aat   435
Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn
                130                 135                 140

gcc aca gtt gaa gac agt gga acc tac tac tgt acg ggc aaa gtg tgg   483
Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp
            145                 150                 155

cag ctg gac tat gag tct gag ccc ctc aac att act gta ata aaa gct   531
Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala
        160                 165                 170

ccg cgt gag aag tac tgg cta caa ttt tag gatccattg                570
Pro Arg Glu Lys Tyr Trp Leu Gln Phe
    175                 180
```

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             20                  25                  30

Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser
         35                  40                  45

Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp
     50                  55                  60

Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro
 65                  70                  75                  80

Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala
                 85                  90                  95

Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp
            100                 105                 110
```

```
Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala
        115                 120                 125

Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr
    130                 135                 140

Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu
145                 150                 155                 160

Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg
                165                 170                 175

Glu Lys Tyr Trp Leu Gln Phe
            180
```

Claims

1. Recombinant soluble Fc receptor
characterized by the absence of transmembrane domains, signal peptide and glycosylation.

2. Recombinant Fc receptor according to claim 1,
wherein the receptor is a FcγR or a FcεR.

3. Recombinant Fc receptor according to claim 1 or 2,
wherein the receptor is a FcγRIIb.

4. Recombinant Fc receptor according to any one of claims 1 to 3,
wherein the receptor is of human origin.

5. Recombinant Fc receptor according to any one of claims 1 to 4,
wherein it contains the amino acids as shown in SEQ ID NO: 1 or SEQ ID NO:2.

6. Recombinant nucleic acid containing a sequence encoding a recombinant Fc receptor according to any one of claims 1 to 5.

7. Recombinant nucleic acid according to claim 6,
wherein it contains a sequence as shown in SEQ ID NO:3 or SEQ ID NO:4.

8. Recombinant nucleic acid according to claim 6 or 7,
wherein it additionally contains expression control sequences operably linked to the sequence encoding the recombinant Fc receptor.

9. Recombinant nucleic acid according to any one of claims 6 to 8,
wherein it is contained on a prokaryotic expression vector, preferably a pET vector.

10. Host cell characterized by the presence of a recombinant nucleic acid according to any one of claims 6 to 8.

11. Host cell according to claim 10,
wherein it is a prokaryotic host cell, preferably an E. coli cell.

12. Process for the determination of the amount of antibodies of a certain type in the blood, plasma or serum of a patient, characterized by the use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 in an immunoassay and determination of the presence of FcR-antibody complexes.

13. Process according to claim 12, wherein the immunoassay is an ELISA and preferably a sandwich assay.

14. Process according to claim 12 or 13, wherein the antibodies to be determined are IgE antibodies and the recombinant soluble receptor is a FcεR.

15. Process according to claim 14 for the determination of a predisposition or manifestation of an allergy.

16. Process according to claim 12 or 13, wherein the antibodies to be determined are IgG antibodies and the recombinant soluble receptor is a FcγR.

17. Process for the determination of the immune status of patients with chronic diseases of the immune system, wherein a Fc receptor according to any one of claims 1 to 5 is used in a competitive immunoassay and the amount of the corresponding sFcRs in the blood, plasma or serum of a patient is determined.

18. Process according to claim 17, wherein the chronic disease is AIDS, SLE, MM or rheumatoid arthritis.

19. Use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

20. Use according to claim 19, wherein recombinant soluble FcγRs are used and recognition and binding of IgG antibodies is of interest.

21. Pharmaceutical composition containing as active agent a recombinant soluble FcR according to any one of claims 1 to 5.

22. Pharmaceutical composition according to claim 21 for use in the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

23. Pharmaceutical composition according to claim 21 or 22 for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma, containing a recombinant soluble FcγR preferably having the amino acid sequence as shown in SEQ ID NO:1.

24. Use of a crystalline preparation of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the generation of crystal structure data of Fc receptors.

25. Use of crystal structure data obtained by the use according to claim 24 for the identification and preparation of Fc receptor inhibitors.

26. Use of crystal structure data obtained by the use according to claim 24 for the identification and preparation of new antibody receptors.

27. Use according to any one of claims 24 to 26 in a computer-aided modelling program.

28. FcR inhibitor characterized in that it has a three-dimensional structure which is complementary to the recombinant soluble FcR according to any one of claims 1 to 5.

29. Pharmaceutical composition containing as active agent a FcR inhibitor according to claim 28.

30. Pharmaceutical composition according to claim 29 for use in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system.

31. Pharmaceutical composition according to claim 29 or 30 for the treatment or prevention of allergies, autoimmune diseases or an anaphylactic shock.

32. Fc receptor according to claims 1-5, bound to a solid phase.

33. Pc receptor according to claim 32, wherein the solid phase is a chromatography carrier material.

34. Use of a chromatography carrier material according to claim 33 for the adsorption of immunoglobulins from the blood, plasma or serum of a patient or from culture supernatants of immunoglobulin producing cells.

35. Use according to claim 34 for the enrichment of antibodies from a patient's blood, serum or plasma or from culture supernatants of immunoglobulin producing cells for the conduction of further tests.

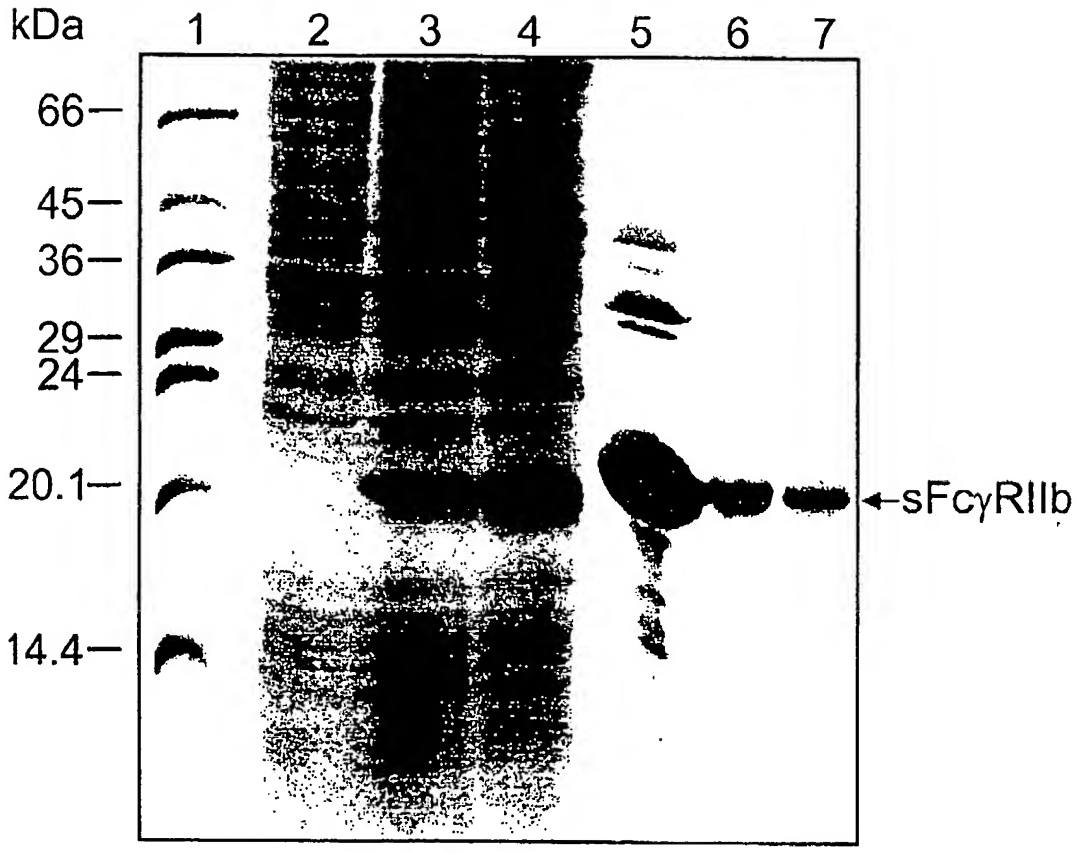


FIG. 1

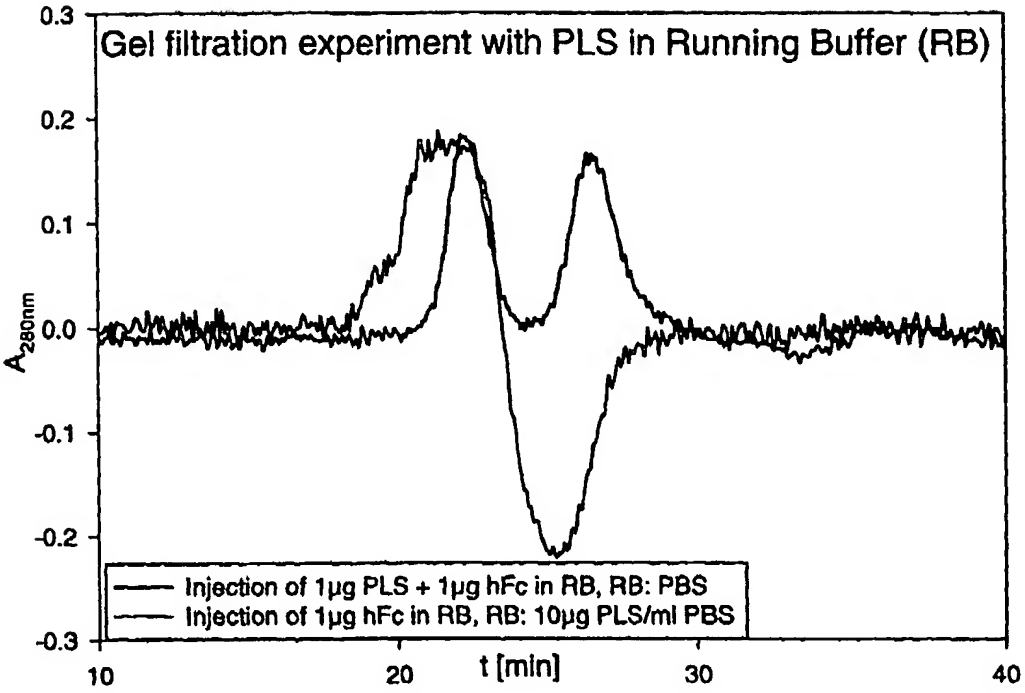
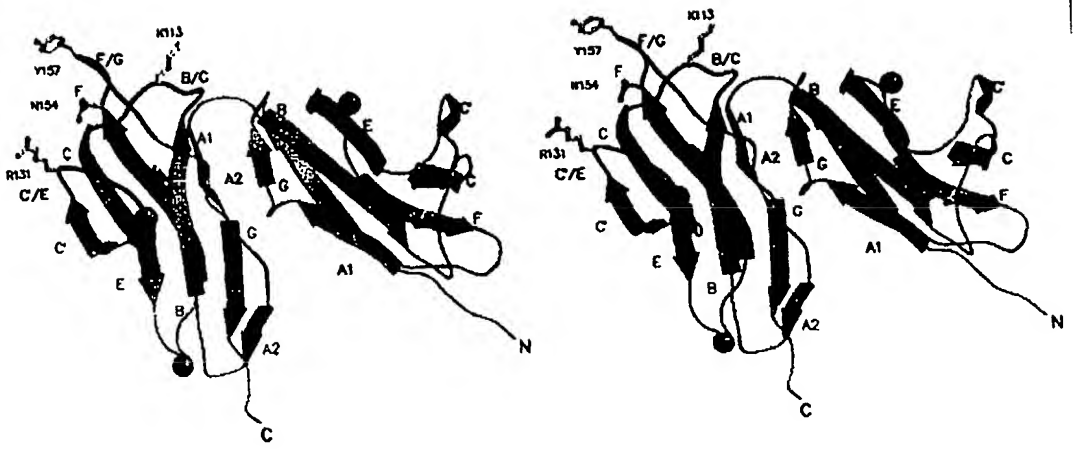

Gel filtration experiment with PLS in Running Buffer (RB)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
A280nm
10
20
30
40
t [min]
Injection of 1µg PLS + 1µg hFc in RB, RB: PBS
Injection of 1µg hFc in RB, RB: 10µg PLS/ml PBS

FIG. 2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F/G
Y157
B/C
F
N154
A1
B
E
C'
C
R131
C/E
A2
G
C
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C'
G
A1
E
B
A2
C
N
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B/C
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C
N

FIG. 3

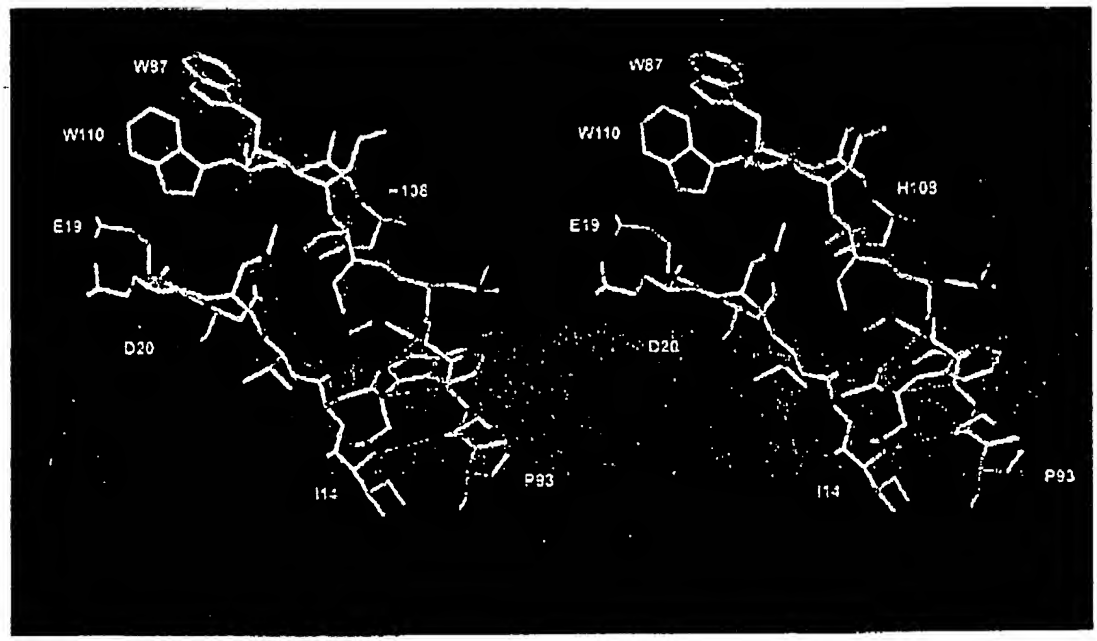

FIG. 4

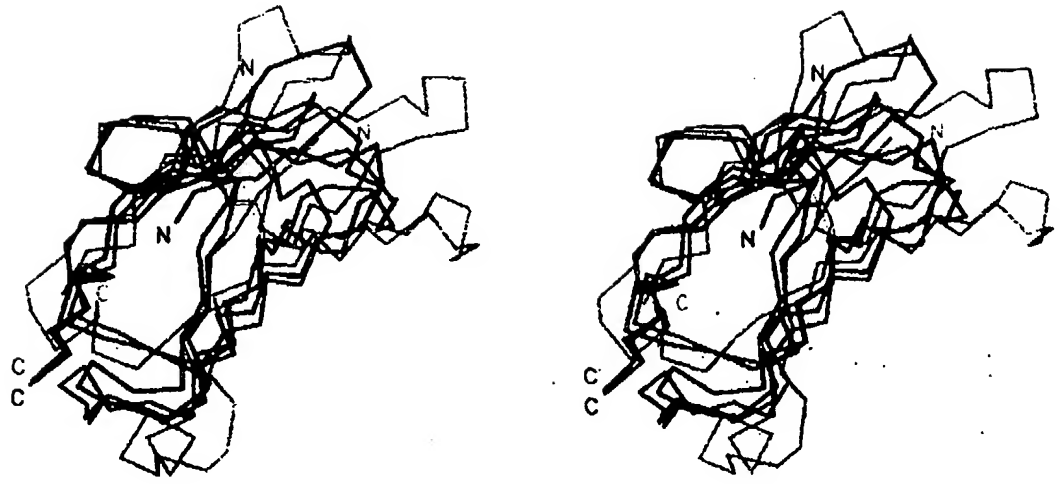

FIG. 5A

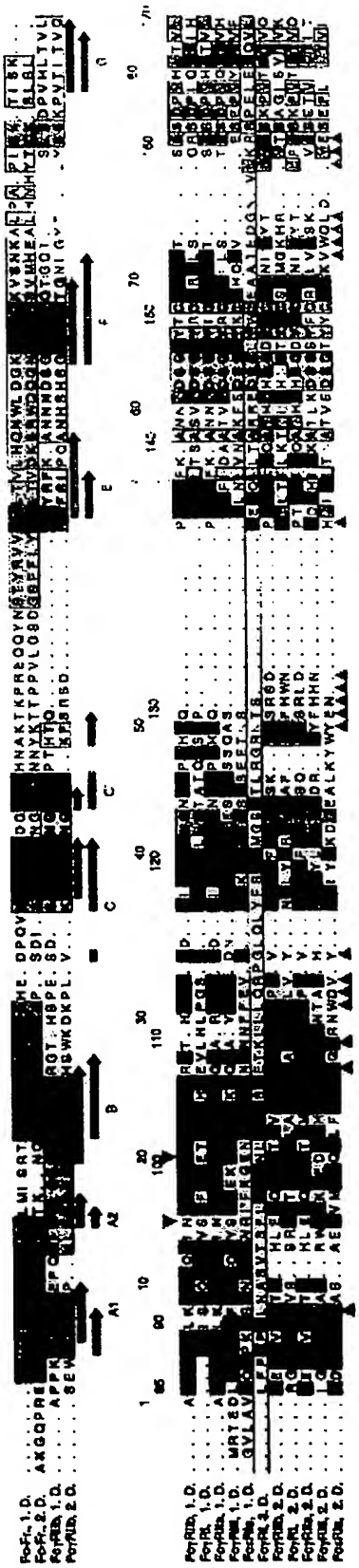

FIG. 5B

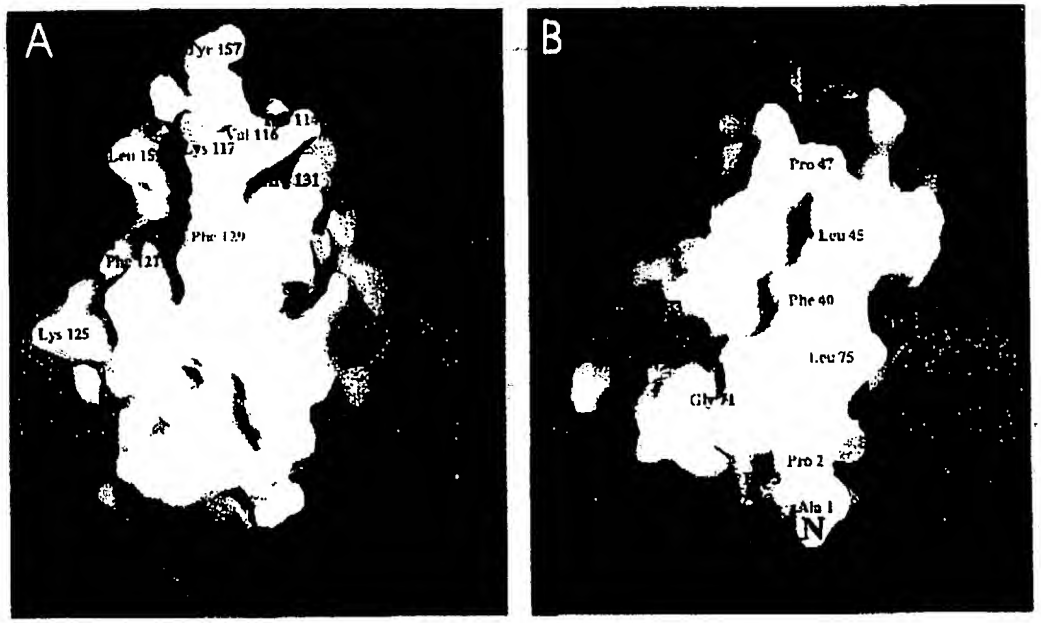
A
Tyr 157
Val 116
Lys 117
131
Phe 129
Lys 125
B
Pro 47
Leu 45
Phe 40
Leu 75
Pro 2
Ala 1
N

FIG. 6

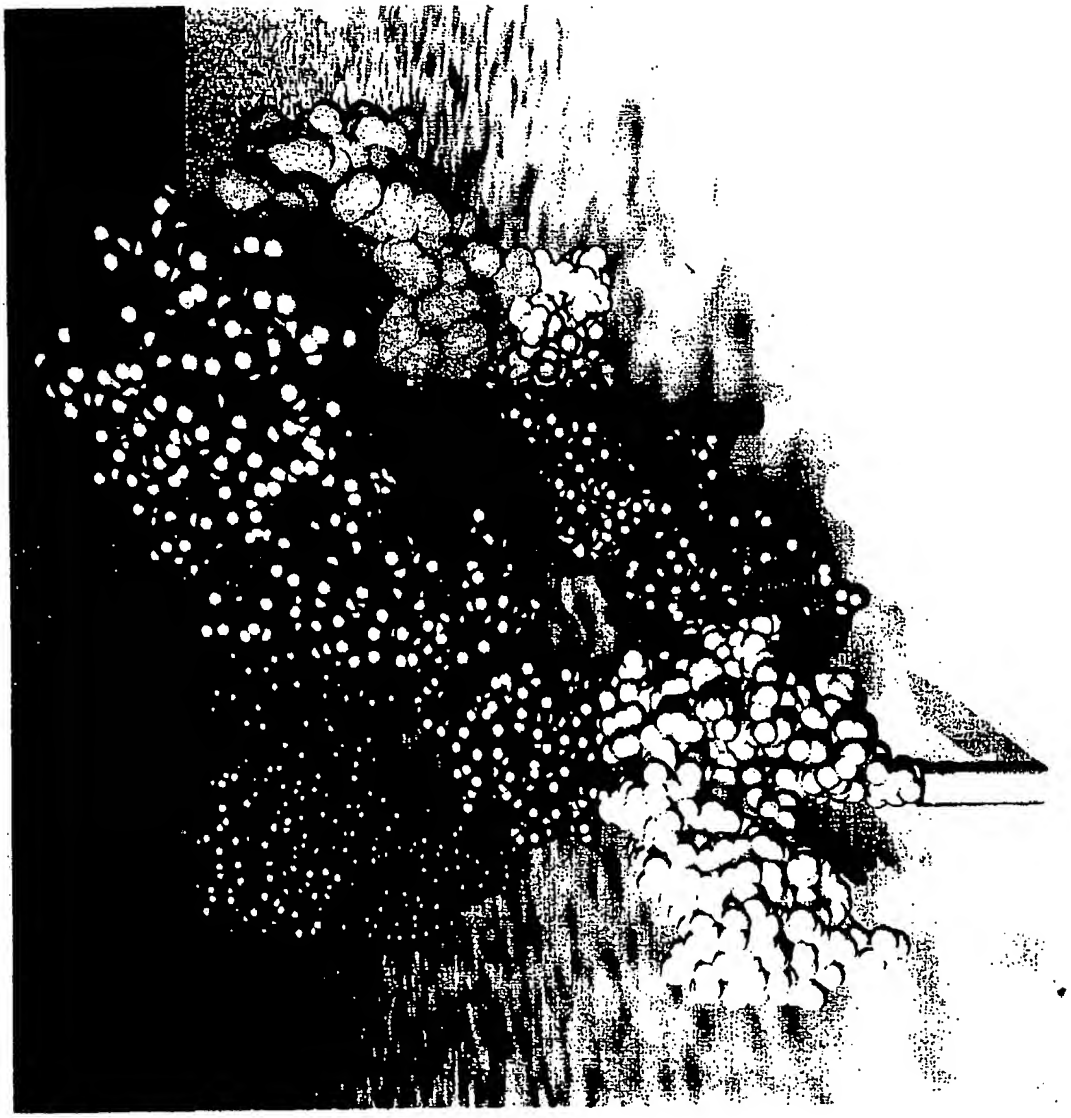

FIG. 7

Alignment of the amino acid sequence of the extracellular parts of FcγR and FcεRIa
(without signal sequence and transmembrane region)

```
          1                                                  50                                                 100
FcγRIIa   SQAAAPPKAV LKLEPPWINV LQEDSVTLTC QGARSPESDS IQWFHNGNLI PTHTQPSYRF KANNNDSGEY TCQTGQTSLS DPVHLTVLSE WLVLQTPHLE
FcγRIIb   GTPAAPPKAV LKLEPQWINV LQEDSVTLTC RGTQSPESDS IQWFHNGNLI PTHTQPSYRF KANNNDSGEY TCQTGQTSVS DPVHLTVLSE WLVLQTPHLE
FcγRIII   MRTEDLPKAV VFLEPQWYSV LEKDSVTLKC QGAYSPEDNS TQWFHNESLI SSQASSYFID AATVNDSGEY RCQTNLSTLS DPVQLEVHIG WLLLQAPRWV
FcγRI     ........AV ISLQPPWVSV FQEETVTLHC EVLHLPGSSS TQWFLNGTAT QTSTPSYRIT SASVNDSGEY RCQRGLSGRS DPIQLEIHRG WLLLQVSSRV
FcεRIa    GVLAVPQKPK VSLNPPWNRI FKGENVTLTC NGNNFFEVSS TKWFHNGSLS EETNSSLNIV NAKFEDSGEY KCQHQQVNES EPVYLEVFSD WLLLQASAEV

          101                                                150                                                200
FcγRIIa   FQEGETIMLR CHSWKDKPLV KVTFFQNGKS QKFSRLDPTF SIPQANHSHS GDYHCTGNIG YTLFSSKPVT ITVQVPSMGS SSPMGII... ..........
FcγRIIb   FQEGETIVLR CHSWKDKPLV KVTFFQNGKS KKFSRSDPNF SIPQANHSHS GDYHCTGNIG YTLYSSKPVT ITVQAP...S SSPMGII... ..........
FcγRIII   FKEEDPIHLR CHSWKNTALH KVTYLQNGKD RKYFHHNSDF HIPKATLKDS GSYFCRGLVG SKNVSSETVN ITITQGLAVS TISSFSPP.. ..........
FcγRI     FTEGEPLALR CHAWKDKLVY NVLYYRNGKA FKFFHWNSNL TILKTNISHN GTYHCSG.MG KHRYTSAGIS VTVKELFPAP VLNASVTSPL LEGNLVTLSC
FcεRIa    VMEGQPLFLR CHGWRNWDVY KVIYYKDGEA LKYWYENHNI SITNATVEDS GTYYCTGKVW QLDYESEPLN ITVIKAPREK YWLQ...... ..........

          201                                                250                       277
FcγRIIa   .......... .......... .......... .......... .......... .......... .......... .......
FcγRIIb   .......... .......... .......... .......... .......... .......... .......... .......
FcγRIII   .......... .......... .......... .......... .......... .......... .......... .......
FcγRI     ETKLLLQRPG LQLYFSFYMG SKTLRGRNTS SEYQILTARR EDSGLYWCEA ATEDGNVLKR SPELELQVLG LQLPTPV
FcεRIa    .......... .......... .......... .......... .......... .......... .......... .......
```

FIG. 8

EP 1 006 183 A1

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 12 2969

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EP 0 614 978 A (ROUSSEL-UCLAF) 14 September 1994 * page 3, line 1 - page 7, line 33; examples * ---	1-3,6, 8-11, 21-23	C12N15/12 C07K14/705 C12N1/21 C12N15/70 G01N33/53 G01N33/68
X	US 5 623 053 A (LOUIS N. GASTINEL ET AL.) 22 April 1997 * column 5, line 60 - column 8, line 67; examples * ---	1,4,6, 8-13, 24-27	A61K38/17 C07K17/00
X	WO 96 40199 A (UNIVERSITY OF PENNSYLVANIA) 19 December 1996 * page 22, line 24 - page 24, line 14; claims 13-16,36-39; examples I,II * ---	1,2,4,6, 8-16, 19-22, 32-35	
X	WO 95 09002 A (UNIVERSITY OF PENNSYLVANIA) 6 April 1995 * page 15, line 4 - page 16, line 22; claims 28-31; examples I,II * --- -/--	1,2,4,6, 8-13,16, 21,32,33	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
C12N
G01N
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

28-31

Reason for the limitation of the search:

Subject-matter directed to inhibitors has not been searched due to the lack of adequate technical description thereof in the application.

Place of search	Date of completion of the search	Examiner
THE HAGUE	6 May 1999	Montero Lopez, B

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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EPO FORM 1503 03.82 (P04C07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 12 2969

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